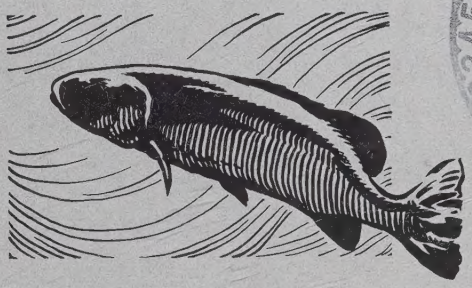
Vol. 18 No. 3 JULY 1956

THE

Progressive Fish-Culturist



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The printing of this publication has been approved by the Director of the Bureau of the Budget, April 8, 1954.

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THE

Progressive Fish-Culturist

A Quarterly for Fishery Biologists and Fish-Culturists

Volume 18 | Washington 25, D. C. | July 1956 | Number 3

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SURVIVAL OF FISH SPERM AFTER FREEZING AND STORAGE AT LOW TEMPERATURES

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and
Howard P. Clemens, University of Oklahoma, Norman, Oklahoma

THE PRACTICAL POSSIBILITIES FOR THE USE OF STORED FISH SPERM are evident to any fish culturist. Sperm from desired species could be shipped to other hatcheries. The number of males kept from season to season could be reduced or possibly eliminated altogether at some hatcheries. Selective breeding of desirable strains of fish could be effected even after the males were dead. If males ripened in advance of females, sperm could be stored until needed.

Techniques used in storing semen at low temperatures were discussed by Mann (1954) in his review of the physiology and biochemistry of semen. Some of the more successful attempts to store fish milt are those of Rucker (1949), who preserved the fertilizing capacity of salmon (Oncorhynchus nerka) milt for 14 days in a refrigerator; Butcher (1944), who stored brown trout (Salmo fario) milt covered with paraffin 5 days at a temperature of 2.2° to 3.3° C.; and Smith and Quistorff (1943), who kept steelhead trout (S. gairdneri) and chinook salmon (O. tshawytscha) milt 2.5 to 5 hours at temperatures of 6° to 7° C.

The possibility of freezing the sperm of any species was first brought into prominence by Polge, Smith, and Parkes (1949), who demonstrated that fowl sperm could withstand low temperatures when treated with a Ringer-glycerol solution. When one volume of fowl semen was mixed with one volume of 40 percent glycerol in Ringer's solution, frozen, and cooled to -79° C., the sperm resumed motility when rapidly thawed. This glycerol method was applied to bull semen (Smith and Polge 1950), and cows were inseminated successfully with sperm that had been frozen and stored by this method (Polge and Rowson 1952). Equally good results were obtained with human sperm (Bunge and Sherman 1953). Practically all of the recent techniques for storing sperm have used glycerol in various concentrations, and investigations have involved the selection of suitable extenders (diluters) and the optimum rate of cooling and thawing. In the selection of extenders, particular attention has been given isotonicity, ion quality, nutrition, chemicals of bactericidal value, and the properties that exhibit a protective influence.

We began our experiments with carp and goldfish milt, which, we have learned, can be obtained by hand-stripping in practically any month of the year. We found that sperm taken from fish prior to natural emission became extremely motile when diluted with water and was, so far as microscopic examination revealed, as normally motile as the sperm taken at

This investigation was conducted under the Saltonstall-Kennedy Act for commercial fisheries research, University of Oklahoma Research Institute Project 120, Contract No. 14-19-008-2382. Dr. Carl D. Riggs, Project Leader, directed the work.

The authors are entitled to equal credit for this investigation.

spawning time. This milt was collected as free as possible of urine, fecal matter, blood, and water, and was immediately added to the various extenders in 1-milliliter vials equipped with corks.

A Suitable Extender for Fish Milt

A major step toward successful freezing and cold storage of fish milt was made when we discovered that frog Ringer's solution (containing NaCl, 650 mg.; KCl, 14 mg.; $CaCl_2$, 12 mg.; $NaHCO_3$, 20 mg.; NaH_2PO_4, 1 mg.; and glucose 200 mg. per 100 ml. of solution) was a suitable medium in which sperm from out-of-season carp (sperm collected at times other than normal spawning season) could be kept immotile for 30 days when stored at 3° to 5° C. When diluted with tap water, these sperm became normally motile. After about 10 days of storage, there was a progressive decrease in the number of motile cells, and this loss was proportional to the time of storage. The factors that apparently limited longer storage were the growth of bacteria and the evaporation of seminal plasma, especially in the undiluted specimens. The addition of 1,000 units of crystalline penicillin per milliliter of diluted milt interfered with sperm motility and did not appreciably prevent the growth of bacteria.

Different results were obtained when out-of-season carp were injected (February 14, 1956) with 2 to 3 milligrams of freeze-dried pituitary material from the black buffalo (Ictiobus niger) 24 hours prior to stripping. The milt obtained contained sperm which were not inactivated by frog Ringer's solution. At first, we thought the activity of the sperm might be due to the direct effects of injected hormones. This possibility was minimized when uninjected goldfish which had been transferred from winter ponds to the laboratory 1 month previously and had been kept at 72° F. produced sperm which were active in frog Ringer's solution. When 200 to 500 milligrams of potassium chloride were added to 100 milliliters of frog Ringer's solution, the sperm from ripe fish were immotile. The amount of potassium chloride varied in accordance with the degree of ripeness of the donor fish. This immotility may be related to ion quality, to the osmotic balance of the storage medium, or to the interaction of these two factors. The exact relationship of potassium to fish sperm motility was not demonstrated during this investigation.

Other extenders were also tried. A phosphate buffer with a pH of 7.4 (2.0 gm. $Na_2HPO_4 \cdot 12H_2O$; 0.2 gm. KH_2PO_4 per 100 ml. of H_2O) plus 6 percent glycerin usually produced immotility of out-of-season carp sperm, but not of sperm that had been obtained from fish ripened by the injection of dried pituitaries. A solution of 3 percent sodium citrate dihydrate ($Na_3C_6H_5O \cdot 2H_2O$), with a pH of 7.6 gave the same results.[1/]

When ripe sperm were stored at 3° C. in the phosphate and the citrate extenders containing 6 percent glycerin, many cells were motile each time a sample was examined under the microscope, even after periods of storage exceeding 1 week. This motility may have been initiated when the sample was placed on the slide. Possibly this motility was due to the fact that the sperm were exposed to greater oxygen tensions, a relationship which has been demonstrated for sea-urchin semen (Mann 1954: 74-75). A closer examination of this motility revealed that some sperm cells became motile and then rested while cells that had been resting became motile. Such intermittent motility on the slides continued for as much as 1 hour, or until significant evaporation of fluid had taken place. The cells swelled very little when they became motile, and the fact that they were capable of becoming highly motile after coming to rest was demonstrated by the addition of water to samples which had reached a state of inactivity.

1/ These buffers are widely used as diluters for bovine sperm. For advice concerning these extenders and others, we are grateful to Dr. G. T. Easley, Turner Ranch, Sulphur, Oklahoma.

Effective Concentrations of Glycerol for Sperm Survival

Tests were conducted to determine the effect of various concentrations of glycerin on carp sperm. Samples (less than 0.1 milliliter) of carp milt were added to 0.2 milliliter of frog Ringer's solutions containing 1 to 30 percent glycerin. Another series was made in which the glycerin concentration was increased stepwise from 3 to 30 percent by steps of 3 percent; i.e., one volume of 9 percent glycerol-Ringer's was added to one volume of milt and 3 percent glycerol-Ringer's to give a final concentration of 6 percent glycerin, and thus up to 30 percent concentrations. Between steps the samples were centrifuged and the supernatant was discarded. This technique kept the sperm count high and prevented overflowing of the vials. The elimination of the seminal plasma in this process may be highly undesirable, as the sperm cells may rely on the medium for metabolism. Nevertheless, many of these samples contained motile sperm after such treatment, both before and after freezing.

In general, these tests indicated that sperm could be placed directly into a concentration of 6 percent glycerin in Ringer's solution without impairment of motility, and that little motility could be obtained if the sperm were placed directly into 10 percent or higher concentrations of glycerin in Ringer's. However, when the glycerin was added stepwise, some motility could be elicited from all concentrations up to 30 percent.

High concentrations of glycerin may be deleterious to the sperm in a manner that upsets the water balance of the cell, so that when water is added to the medium, the resultant swelling damages the sperm cell before motility is initiated. We believe that this hypothesis has merit, for great care had to be exercised in making the water dilutions in all the glycerin concentrations in excess of 9 percent. Motility was observed only when a small drop of water was added to a drop of sperm suspension in such a way as to produce an area of graded dilutions between the suspension and the tap water. Apparently only those cells which were diluted exactly at the optimum rate became motile. Those cells which received too much water swelled very quickly and did not move. Attempts to increase the number of motile cells by stepwise dilutions of the high glycerin concentrations met with little success. Dialysis through cellophane against isotonic Ringer's may produce better results, but this was not tried.

Freezing Experiments

Carp milt was added to various concentrations and combinations of frog Ringer's, sodium citrate, and phosphate buffer. Identical solutions, with the addition of various concentrations of glycerin, were used. Cane sugar and fresh egg yolk were tried, alone and in combination with glycerin, as protective agents for fish sperm. The milt was collected and extended at room temperature. In each series, three or more identical solutions were prepared, and each of these was frozen in a different manner. Some samples were slowly cooled to -15° C. and then were thrust into a dry-ice chest at a temperature of about -73° C. Other samples were placed directly into the dry-ice chest from room temperature or from refrigerator temperature (2° to 5° C.). Some of the solutions were frozen immediately after the addition of the diluters, and others were allowed time to equilibrate after dilution or between additions of increasing concentrations of glycerin. The frozen samples were thawed at various intervals by immersing the vials in tap water or by placing them on the laboratory desk. A few specimens were thawed more slowly when placed in progressively higher temperatures in the refrigerator.

From more than two hundred samples, a number of observations were drawn: (1) No sperm survived freezing in pure seminal plasma or in any of the extenders without the addition of either glycerol or sugar; glycerol was by far the more effective. (2) The greatest number of motile cells was found in milt frozen in Ringer's plus 6 percent glycerin, or plus 12 percent

glycerin when added in three steps. Samples frozen for 5 to 10 minutes exhibited an estimated 75 percent motility when thawed and diluted with water, but fewer cells were motile with increased storage time. After 60 hours of storage, about 20 percent of them were motile when thawed. (3) In samples which contained either sodium citrate or the phosphate buffer, the sperm cells clumped and sometimes the seminal plasma coagulated when frozen. In spite of this coagulation, some cells (about 5 to 15 percent) were motile after being frozen in these solutions which contained 6 to 7 percent glycerin. These results were not significantly changed when egg yolk was added. (4) Five percent cane sugar in Ringer's solution offered slight protection in freezing, either when combined with glycerin or alone; but higher concentrations were detrimental. (5) There was an indication that slow freezing (1 to 2 hours) to -15° C. before storage at -73° C. was beneficial. (6) Slow thawing may be beneficial. (7) There were only insignificant differences between samples which were allowed to equilibrate for 15 minutes to 24 hours after the addition of solutions and those samples which were frozen immediately.

Discussion

Since the carp milt can be frozen and stored at a temperature of -73° C. in isotonic solutions of salts plus 6 to 12 percent glycerol, and since 20 percent of the sperm survived after 60 hours of storage, it may be possible to develop solutions and techniques which permit much better survival. In fact, these experiments suggest that results depend as much on the tonicity and composition of the extender as on the protective action of glycerol. It is especially apparent that the duration of storage at low temperatures depends upon the perfection of the diluting medium. We do not yet have evidence that frozen fish sperm will fertilize eggs, but the possibility is suggested by the literature on other animals, notably cattle and man.

Summary

1. Normal frog Ringer's solution was isotonic to sperm from out-of-season carp and goldfish, and it maintained sperm inactivity. A few sperm were capable of motility after storage in these solutions at 5° C. for 30 days.

2. It was necessary to add 200 to 500 milligrams of potassium chloride to normal frog Ringer's to produce immotility in sperm from ripe fish, whether the ripening process was accelerated by temperature or by injections of dried pituitaries.

3. Carp milt diluted with isotonic Ringer's plus 6 to 12 percent glycerin, frozen, and stored at a temperature of -73° C. for 60 hours contained 20 percent motile sperm when thawed.

4. Complete survival of frozen sperm and storage for long periods probably depend upon perfection of diluters.

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Though artificial propagation of frogs on a commercial basis has not been proved successful, it has been suggested that the raising of muskrats and frogs might be combined to advantage.

POOR MAN'S PORTABLE SCALE PRESS

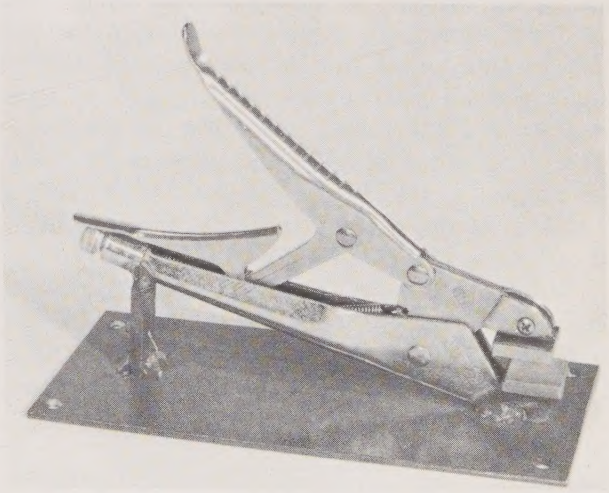
To meet a long-recognized need in the field, James M. MacMartin of the Vermont Fish and Game Service has devised an inexpensive scale press that is readily carried from one point to another.

"While we were looking around to find some suitable tool which could be adapted to this purpose," writes Mr. MacMartin from his office in Montpelier, "it was thought that perhaps a 'vise-grip' type of tool could be used. The particular tool which eventually was modified to meet our requirements was a snap-locking wrench."

As originally designed, this lever-jaw wrench had one toggle-mounted jaw. For its use as a scale press (see photograph), the toggle-mounted jaw and the opposing stiff-mounted jaw have been replaced with two flat-ground steel plates, 1-inch square. The action of the toggle mount is to bring the two plates parallel to each other as pressure (2,000 pounds to the square inch) is applied. The release lever, which extends beyond the tail of the upper handle, permits one-handed action when it is desired to take material from the press. Very satisfactory impressions can be made with acetate slips of ordinary weight.

The plate on which the tool has been mounted, though not interfering with its portability, makes it easy to clamp or screw the press to a bench.

"The initial cost of the basic tool was $2.75," concludes Mr. MacMartin, "and added to this initial cost was the charge for several hours of machine-shop work necessary to complete the modification. Thus the total cost to us was about $18 per unit."



(Photograph courtesy of Mr. MacMartin)

THE CHEMISTRY OF DEVELOPING BROWN TROUT EGGS

Arthur M. Phillips, Jr., U. S. Fish and Wildlife Service
Donald R. Brockway, New York Conservation Department
and
George C. Balzer, Jr., U. S. Fish and Wildlife Service
Cortland, New York

ALTHOUGH MANY THEORIES ABOUT PHYSICAL AND CHEMICAL CHANGES IN TROUT EGGS DURING DEVELOPMENT have been advanced through the years, little research has been done to establish these theories as facts. As in other husbandries, establishment of the chemical changes of developing trout eggs can be invaluable in determining the nutritional and physiological requirements of adult trout.

Hayes (1930, 1930a) reported that the wet weight of salmon eggs increased during incubation; that the protein, after an initial decrease, increased; and that the fat content of the egg first increased and then decreased as the hatching period approached. Hayes' work was based on the later stages of development after the embryo was well formed. Lovern (1934, 1936) stated that the fat content of salmon eggs was a reflection of the fat in the mother's body, and that the characteristics of the egg fat changed as the egg developed. He reported that the iodine number of the egg fat shifted from a high of 206 to a low of 176 just before hatching.

Very little work on trout eggs has been reported, though it is commonly believed by fishery workers that wet weight of trout eggs increases as development progresses.

Procedure

To determine the chemical changes that occur in brown trout eggs during incubation, a series of samples was made from a single lot of eggs, samples being taken at weekly intervals from the water-hardening process through the hatching period. The samples consisted of 2 groups of 300 eggs, which were weighed before being preserved in 95 percent ethyl alcohol. After being dried to constant weight to determine water content, the samples were analyzed for protein, ash, fat, and the iodine number of the fat.[1/]

Because no other adults were available for these studies, the eggs were taken from 3-year-old brown trout that had been subjected to light control to induce early spawning (which occurred approximately 1 month earlier than normal).

The eggs were in the eyed stage by the fourth week and hatched by the sixth; in the seventh week the samples consisted of week-old sac fry.

The results are summarized in the table and the chart. All data are averages of duplicate analyses of the duplicate samples.

Chemical Changes

Water. --The percentage of water in the developing egg decreased until the end

[1/] Analytical methods: Protein, micro-Hengar; Ash, ignition to constant weight (muffle furnace); Fat, continuous ether extraction; Iodine number of the fat, Hanus.

CHEMICAL ANALYSES OF DEVELOPING BROWN TROUT EGGS (WEEKLY INTERVALS)

Time in weeks	Total weight sample (grams)	Dry basis			Wet basis				Fat iodine number
		Percentage of--			Percentage of--				
		Protein	Ash	Fat	Water	Protein	Ash	Fat	
0 ----------	17.3	72.3	3.87	11.7	68.1	23.1	1.23	3.7	134
1 ----------	17.1	74.4	3.84	10.3	67.7	24.0	1.14	3.4	148
2 ----------	16.1	73.8	3.83	9.2	66.4	24.7	1.29	3.1	139
3 ----------	16.1	72.6	3.75	7.9	65.8	24.8	1.28	2.7	157
4 (eyed eggs)--	15.9	74.3	4.08	7.6	67.3	24.2	1.43	2.5	152
5 ----------	15.9	67.8	3.63	9.0	64.0	23.9	1.29	3.1	138
6 (hatching)---	15.9	70.3	3.88	10.9	68.3	22.3	1.23	3.4	137
7 (sac fry)----	20.1	68.5	4.40	12.7	73.9	17.3	1.14	3.3	150

of the fifth week, after which the water content increased rapidly. The newly hatched fry had considerably more water than the water-hardened eggs.

Protein.--The protein content of the egg increased gradually until the third week and then decreased throughout the remainder of the incubation period. The newly hatched fry contained the lowest level of protein found in these studies.

Ash.--The ash content of both eggs and newly hatched fry was constant throughout the period covered by these analyses.

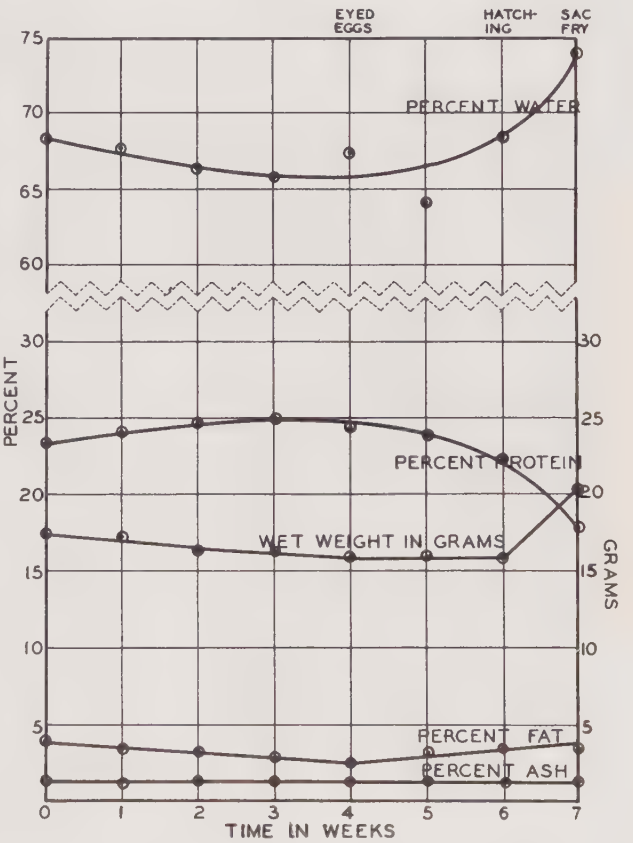
Fat.--The fat content of the eggs decreased during the first 4 weeks and then increased during the remainder of the experimental period.

Iodine number of the fat.--The iodine number of the fat did not change throughout the experimental period.

Wet weight of eggs.--There was a gradual decrease in the wet weight of the eggs during the first 3 weeks of incubation; in the following 3 weeks, the weight was constant. After hatching, the fry showed a marked increase in wet weight.

Discussion

The initial increase in protein content of the egg may be correlated with the decrease in water content. The relatively constant level of the protein during this period on a dry basis supports this correlation (see table). The initial decrease in fat content indicates that fat is burned to meet the energy requirement of the developing embryo. After the third week the catabolism of the egg apparently has shifted. The protein decrease and the fat increase indicate the presence of a surplus of protein, so far as tissue development is concerned. The excess protein



A graphic summary of the chemical analyses of developing brown trout eggs.

may be metabolized to fat within the embryo. The same trends in protein and fat contents of the egg are apparent in the analytical results expressed on a dry basis (see table). Under these circumstances, the changes in fat and protein contents of the egg cannot be correlated with the increase in water content observed for the same time intervals. It is surprising that the ash content remains constant throughout incubation. A mineral uptake from the water has been shown in developing salmon eggs. Hayes, Darcy, and Sullivan (1946) reported that there was an increase in the calcium content of salmon eggs that could not be accounted for by the calcium present in the yolk of the egg. If a mineral uptake occurs in these studies, it is accompanied by a similar loss, as the total ash fails to show any marked change throughout the period of development.

In the chart, all of the plotted points fit the hand-drawn curves, with the exception of the curve for water content. The amounts of water within the egg at the fourth and fifth weeks do not fall on the curve that is drawn. This appears as an analytical error, but duplicate analyses of the 2 samples of eggs (300 eggs in each sample) are in reasonable agreement (67.9 and 66.7 percent the fourth week, and 63.3 and 65.8 percent the fifth week). There is a possibility that these 2 points are as plotted, and that they reflect the previously hypothesized change in catabolism of the developing egg. The shift in the protein and fat contents of the developing eggs occurs at about the same time interval (the fourth and fifth weeks). This indicates that the maximum need of the embryo for protein occurs at this stage of development. The excess protein may be deposited as fat and/or burned for energy, both processes forming nitrogenous wastes, accounting for the increase in water to dilute the waste products, followed by a decrease in water after equilibrium is established.

Some of these observations are not in agreement with accepted beliefs or published reports. The wet weight of brown trout eggs decreases to a constant level during incubation instead of increasing--as is commonly believed, and as Hayes (1930) reported in his study on salmon eggs. The data in Hayes' report do not agree with his conclusions. The wet weight of the entire egg does not increase significantly, but the wet weight of the embryo does. It seems likely that an error has caused the replacement of the word "embryo" with the word "egg." The changes in both fat and protein content of the eggs during incubation in the present studies are contrary to the conclusion of Hayes (1930a). These deviations are difficult to explain except through the possibility of species difference or through changes brought about by light control of the adult trout to induce early spawning. Hayes worked with wild fish, and the present studies deal with hatchery trout. Whether the diet of wild salmon (natural food) and the diet of hatchery trout (hatchery foods) have an effect, is not known. Because Hayes worked only with the later stages of egg development, it is possible that he would have drawn different conclusions if he had studied the entire incubation period.

An interesting implication of these studies is the change in the egg metabolism that is indicated during incubation, as well as the possible effect that the diet of the mother may exert upon the storage of material within the egg, thus influencing the normal metabolism of development.

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A DEVICE FOR EMERGENCY AERATION OF A REARING POND

Occasionally there is urgent need to aerate a rearing pond with as little delay as possible. To meet such an emergency, D. C. Haskell and L. G. Bouchard, employees of the New York State Conservation Department, have devised a means of quickly raising the oxygen content in water flows of 500 to 800 gallons per minute by 4 to 5 parts per million.

The procedure that has been devised by Messrs. Haskell and Bouchard consists of replacing the air atmosphere over the waterfall at the inlet of a pond with an atmosphere of nearly pure oxygen. This replacement is effected by means of a metal barrel cut in such a way that it will hang on the top dam board (see photograph). The ends of the barrel have been cut to fit tightly on the dam board, and the barrel has been placed so that it dips into the water above and below the dam board to provide a water seal at all points. Oxygen from a cylinder is admitted through the three-quarter-inch threaded hole in the end of the barrel. The falling water draws bubbles of oxygen down into the pond water. The position of the barrel on the dam board is such that in ponds having an 8- to 10-inch fall, most of the bubbles of oxygen are caught again in the barrel as they rise to the water surface; thus the recirculation of the oxygen permits better utilization.

In an aeration system in which bubbles of oxygen are caught and recirculated, nitrogen from the water rapidly dilutes the oxygen. Consequently, it becomes necessary to supply continuously an excess of oxygen, which, in turn, causes gas to leak continuously through the water seals of the barrel. By constantly replacing the oxygen in the barrel, however, it is possible to prevent a buildup of the nitrogen. During tests, a 220-cubic-foot cylinder of oxygen lasted 2 to 4 hours.

This work of Haskell and Bouchard has been part of a continuing study of pond aeration, conducted at the Rome (New York) fish hatchery, with Federal Aid in Fish Restoration funds, as part of Dingell-Johnson Project F-11-R.

Aeration device, as hung on the top dam board at the end of a pond. Water flow is from left to right. Oxygen is introduced through 3/4-inch nipple in right end of barrel.
(Courtesy of Messrs. Haskell and Bouchard)

HISTOPATHOLOGY OF FISH
IV. A GRANULOMA OF BROOK TROUT

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IN THE SUMMER OF 1952, Snieszko and Griffin (1955) diagnosed kidney disease in brook trout from the Fish and Wildlife Service's station at Berlin, New Hampshire. During the examination of these fish, a peculiar lesion was observed in the vicinity of the gastric caeca. In very advanced cases, hard, glistening, white masses of tissue bearing a striking resemblance to mature testes often filled the abdominal cavity. In the initial examinations, the material was actually mistaken for normal testicular tissue. Subsequently, it was recognized as an entirely aberrant, proliferating tumor-like mass.

Further studies revealed that the large masses of tissue apparently started as small papillae from the walls of the stomach. From this point, the disease spread to almost every organ of the body, although the greatest concentration of it usually appeared in the vicinity of the gastric caeca. Histologically, the condition strongly resembled tuberculosis and the mycotic granulomas of mammals.

In a later investigation (Wood et al. 1956), the disease was described in three eastern hatcheries: Berlin, New Hampshire; Leetown, West Virginia; and Cortland, New York. Occurrence of kidney disease at the first two stations suggested a relationship between the two conditions. A subsequent outbreak of kidney disease at Cortland considerably increased the significance of this relationship. Recent observations, however, indicate that the simultaneous occurrence of the two diseases was probably, in part, coincidental.

In the past 2 years, the granuloma has been observed in brook trout from Erwin, Tennessee; Lamar, Pennsylvania; Klamath Falls, Oregon; and Spearfish, South Dakota. Kidney disease has not been diagnosed at any of these stations.

At this point, it was recognized that the granuloma was very similar to a disease described at Cortland 12 years previously by Young and Olafson (1944). These workers diagnosed the lesions as neurilemomas, or tumors of nerve sheaths. Slides from all outbreaks of the disease have now been studied by both groups of workers, and there is agreement that the lesions are strikingly similar.

The disease is probably much more widely distributed than is apparent. The description and illustrations of the pathology should aid in detecting further outbreaks. It is not limited entirely to brook trout, but this species appears far more susceptible than other salmonids. Young and Olafson (1944) described a very mild case of their disease in one brown trout. We have observed slight lesions in three rainbow trout from the station at Leetown.

Pathology

Gross. --The outside wall of the stomach normally presents a smooth appearance. Any pimplelike projections are

FIGURE:

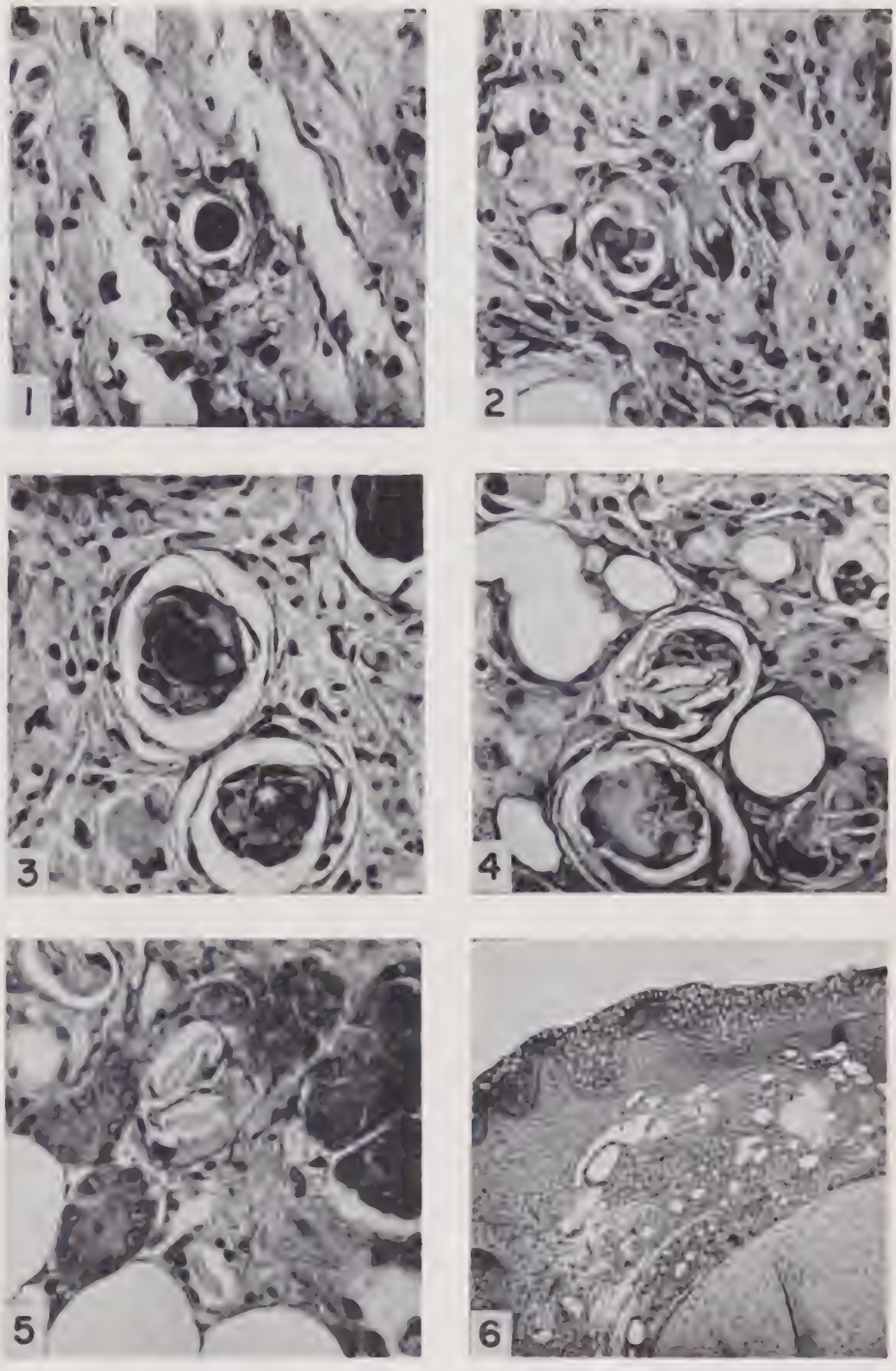
1.--A single mycosislike form. Note the concentric laminations around a central core. x 470.

2.--A cloverleaf form which stimulates budding. x 470.

3.--Forms that show multiple "budding." x 470.

4, 5.--Hyphaelike forms from the Tennessee fish. x 470.

6.--The tongue lesion. The zone between the epithelium and the supporting cartilage (lower right) is pathological. x 48.



indications of the disease in an early stage. White, opaque spots in the fatty tissue around the gastric caeca are highly indicative. These spots are more easily observed after the fish have been preserved in Bouin's solution. As previously described, in advanced cases there may be white, glistening masses of tissue almost filling the abdominal cavity. This material is hard and definitely granular and gritty when cut.

Unfortunately, it appears that the lesions may be extensive inside the wall of the stomach without appearing on the surface. In these cases, it is necessary to resort to microscopic examination.

Microscopic. --The disease has been described as both a tumor and a granuloma. This is not particularly surprising, because both of these conditions may

FIGURE:

7.--Gross appearance of the pimplelike lesions (top) on the exterior wall of the stomach. Normal (bottom). x 4.

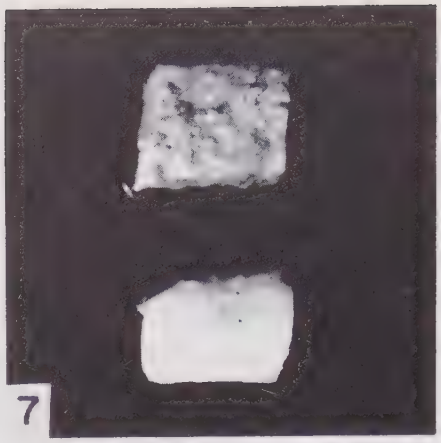
8.--Histological appearance of the stomach lesion. Note the granuloma also in the stomach wall. x 60.

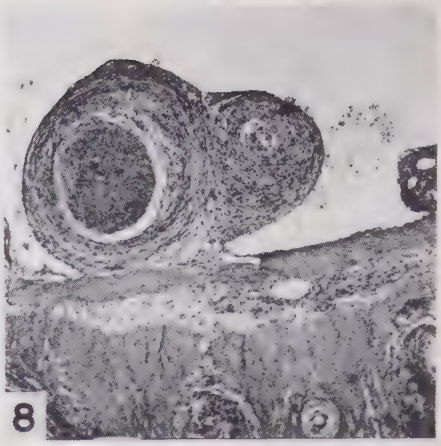
9.--The granuloma from Kearneysville, West Virginia. Stomach. x 100.

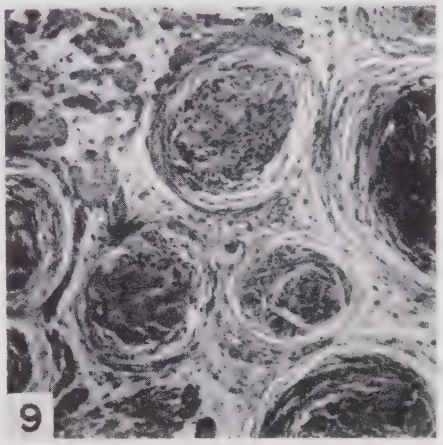
10.--The lesion in spleen tissue from Klamath Falls, Oregon. x 100.

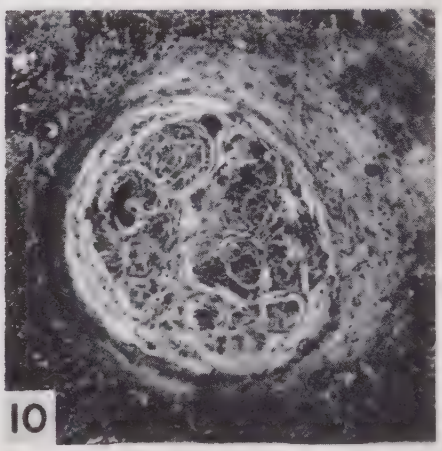
11.--Visceral lesion from Erwin, Tennessee. x 100.

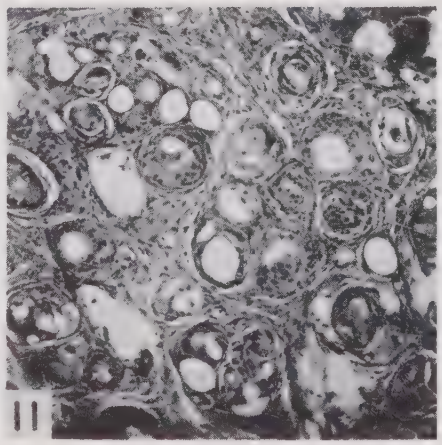
12.--Visceral lesion from Spearfish, South Dakota. x 100.

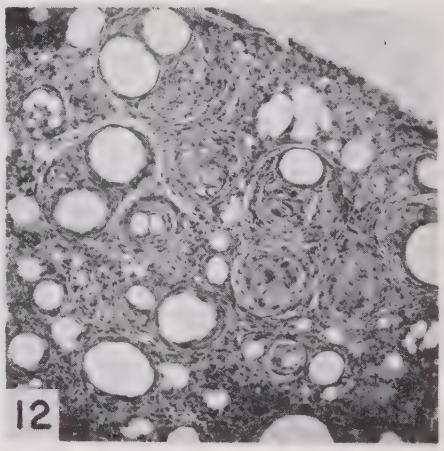












consist of proliferating masses of tissue of nearly identical structure. The lesions are often characterized by circular or somewhat whorled masses of fibrous tissue which frequently resemble both the tubercles of the granulomatous diseases and the whorled structure of nerve-cell tumors. Giant cells are numerous, but these appear to be of the foreign-body type rather than tumorous giant cells.

The lesions have been detected histologically in the walls of the stomach, the adipose tissue surrounding the gastric caeca, the gills, liver, spleen, kidney, eyes, musculature, and tongue. No lesions have been observed in the brain or spinal cord, and none in the intestinal

tract posterior to the gastric caeca. If only mild lesions are present, these lesions are invariably in the muscle wall of the stomach.

Scattered throughout the lesions and frequently inside the tubercles are small structures which resemble mycotic organisms. These oval forms often show laminated concentric lines around a central core. Not infrequently there is seen a cloverleaf type which appears to represent some stage of budding. In earlier material no hyphae were seen, but later specimens appear to show some hyphaelike development.

The lesions are markedly similar in all the material examined from widely different parts of the country. Some specimens show a varying concentration of giant cells, and infected fish from different stations may have some distinguishing characteristics. For example, the New Hampshire and West Virginia samples often show the disease in stages far more advanced than are ever seen from other hatcheries. The Oregon and Pennsylvania fish are affected to a much milder degree, while the South Dakota and Tennessee fish occupy an intermediate position. The Tennessee fish are further characterized by well-developed lesions in the tongue--which are seldom seen in fish from other stations.

Discussion

Two questions are of immediate and practical interest: the cause and the seriousness of the disease.

There is no available information on mortality directly attributable to this disease. It is of significance, however, that in every case but one (Oregon) the disease was observed in tissues submitted for histological examination from fish dying from unknown causes. This does not necessarily indicate that the granuloma was causing the observed mortality. It does signify that a possible relationship exists. The granuloma is a chronic debilitating disease. It may, in itself, cause mortality. It almost certainly lowers the resistance of fish to other pathogens.

Some doubt that the lesions are neurilemmomas has been expressed (Willis 1948). The question is not merely academic, as a granulomatous lesion is more likely to connote an infectious process. However, our attempts to culture an organism and reproduce the disease have been unsuccessful. The structures observed in sections bear only a superficial resemblance to any known mycotic organisms. The Tennessee forms, with hyphaelike structures, show some similarity to the Ichthyosporidium described by Fish (1934) in herring. However, there is very little similarity between most of the stages illustrated by Fish and the present organism. The same appears to apply to the European organism described by Plehn and Mulsow (1911), and by Schaperclaus (1954). It is apparent, therefore, that the etiology of the disease remains almost totally unknown.

The consistent initial appearance of the lesions in the stomach wall suggests this site as the point of entry for the disease. The increased use of dry feed products (fish meals, distillery products, and cereals) in fish diets during the last 30 years provides a possible source for exotic organisms. As yet, there is no evidence to support this hypothesis. In the course of an extensive examination of wild fish, however, we have not observed the disease.

Acknowledgments

It is a pleasure to acknowledge the assistance that has been received from the Fish and Wildlife Service's laboratories at Kearneysville, West Virginia, and Seattle, Washington, and from Dr. W. L. Lehman of the Good Samaritan Hospital, Portland, Oregon.

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TROUT STAMINA

The question of the relative powers of endurance of hatchery trout and wild trout has given rise to innumerable arguments. Recently, however, Norman Reimers, a fishery biologist assigned to California-Nevada Inland Fishery Investigations of the U. S. Fish and Wildlife Service, has reported a "stamina tester" devised at the Service's Convict Creek (California) Experiment Station.

A 20-foot metal trough, with the rectangular open ends screened, was set up as a flume in which the water velocity could be regulated between 0.5 and 3.0 feet per second, and in which the behavior of the trout could be observed. Tests of endurance were then made on 6- to 8-inch fish taken from 3 groups: stream-resident brown trout, rainbow trout that were fresh from the hatchery, and hatchery rainbow trout that had been in the creek for 3 months and had lost much of their plumpness.

For each test, 6 trout from a group were placed in the flume while the water was maintained at a given velocity. The fish were disturbed to keep them swimming, and the times that elapsed between the entrance of the trout into the flume and their appearance, exhausted, at the lower screen were noted. Each group of trout was represented several times at various velocities.

It was noted that in swift water (about 3 feet per second) rainbow trout that had been out of the hatchery just a few days were able to maintain their position only 5 to 10 minutes before collapsing against the screen. The time required for the exhaustion of stream-conditioned rainbow trout averaged a little less than 30 minutes in water at 3 feet per second. On the other hand, wild brown trout resisted the same current a little more than an hour. Similar, though less striking, differences in stamina were observed in trout placed in lower velocities.

The most interesting point in these trials concerned the behavior of the two species in the swift current and again indicated the sagacity of the brown trout as compared with the rainbow trout. In this connection, Mr. Reimers writes:

"...When left undisturbed in the flume, brown trout immediately sought out the dead, or slack, water in the bottom corners of the trough's cross section and remained there without effort, resting on their ventral fins most of the time. Rainbow trout from either group, when they were left undisturbed, continued to weary themselves against the current."

THE NUTRITION OF TROUT
I. GENERAL FEEDING METHODS

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THE HEALTH OF ALL YOUNG ANIMALS depends upon the fulfillment of the physical and physiological requirements for normal growth and development. Many of the factors that control growth are conditions of the environment, and in a controlled environment the efficiency of growth becomes dependent upon the skill and knowledge of the husbandman. In fish hatcheries these factors are numerous. Among the more important are water flow, water temperature, volume of water in the hatchery units, water changes per hour, sanitation, disease, disease-control measures, and lastly--but far from least in importance--the nutrition of the trout.

Nutrition is not solely a matter of diet composition. It is true that a trout cannot grow if essential elements are lacking in the diet, but it is equally true that a complete diet cannot produce fish unless that diet is properly fed. The conversion of food into fish flesh is the measure that is commonly used to judge the efficiency of a feeding program in a hatchery. If the conversion factor is to be regarded as a measure of efficiency, what then can be done to ensure good conversions?

It is planned to discuss, in a series of articles, some of the procedures that a fish culturist can follow in order to improve the efficiency of growing trout in hatcheries. These articles will cover the following topics: General feeding methods, the protein and carbohydrate requirements of trout, the fat and mineral requirements of trout, the vitamin requirements of trout, and ingredients for trout diets.

It would be difficult to determine which of these five subjects has the greatest effect on a hatchery feeding program. In all probability, no one factor is more important than another, and it is a combination of all five that results in an efficient feeding program for the trout hatchery. A common error in feeding is to overemphasize either feeding methods or diet composition to the detriment of the other. The application of all available knowledge, physical and physiological, will result in healthy, fast-growing trout and a low production cost.

GENERAL FEEDING METHODS

Consistency of the Diet

Feeding trout has been rated one of the simplest tasks assigned a fish culturist. It has been a matter of feeding all the fish could consume and then feeding a little more to assure an abundant supply. Consistency of the food has been disregarded; thus, though given more food than necessary, trout have been underfed because the food has been unavailable to them.

Now it is known that the availability of food to the fish is dependent upon the consistency of the diet, and that control of this dietary characteristic alone will result in a lower conversion factor of food into fish flesh. There are several means

of ensuring a maximum intake of food by the trout.

Unbound Diets. --These diets, used for young trout starting to feed, consist of meat alone. The meat is finely ground; the small particles are distributed in the water with a feather, a screen-bottomed dipper, or some other mechanical device. Much food is wasted because the particles soon are lost in the water. What the fish are unable to consume is washed down the drain.

As far as is known now, young trout must be fed in this manner and this loss of food is inevitable until the trout are larger and able to handle more efficient diets. This type of food should be fed for as short a period as possible because of the loss of water-soluble fractions (such as minerals, vitamins, and proteins) during the feeding procedure.

Bound Diets (Mush or Dough Feeds). --At the Cortland Hatchery many years ago, Mr. E. W. Fentress, attempting to mix various meats and meals, discovered that adding salt to spleen results in a rubber-like mass that resists disintegration when placed in the water. Subsequent experiments have established a satisfactory salt level at 2 percent of the diet. These diets prevent the loss of a large portion of the water-soluble nutrients, and the food is more efficient in terms of conversion.

If maximum efficiency is to be obtained from the use of bound diets, several factors must be considered. A spleen binder should be slightly frozen (ice crystals in the flesh) when ground. The best binder with salt is pork spleen. Other binders--named in order of decreasing utility--are beef spleen, horse spleen, beef liver, and beef lungs. Pork and sheep livers bind well, but their use is not recommended. As liver is used for human consumption, it is expensive; also, feeding denatured (dyed) pork and sheep livers can cause excessive losses of fish, apparently through kidney damage resulting from the dye.

Simply binding a diet does not increase conversion efficiencies. The tightly bound and stiff mixture must be properly fed to the fish. There are four satisfactory ways to offer it: First, with a modified potato ricer (Tunison 1945); second, with a pond ricer (Burrows 1947); third, with a compressed-air feeder (Pelnar 1947); and fourth, with a spoon or by means of hand feeding. The food should be given in small particles (not to exceed the size of a dried bean) if the diet is to be utilized by the fish--and not deposited in large lumps on the bottom of the pond. A bound diet is excellent for troughs when it is fed with a modified potato ricer and is satisfactory for ponds when special methods for feeding are employed.

In general, there are two major types of bound diets. First, there is a spleen-liver mixture, with added salt, that is used immediately after the unbound diets. This diet is first fed when the fish reach a size of 3/4 to 1 inch in length. By means of a modified potato ricer with 1/16-inch plate holes, the food is fed as small worm-like particles; these are readily eaten, and there is little loss of nutrients in the water. Second, there is the bound diet that is used when meats and meals are fed to trout that are 1.5 inches and more in length. In both of the diets, the salt and spleen ensure a more complete food consumption, with a resulting increase in production efficiency. In the preparation of bound diets containing meat alone, no water is added. In the preparation of meat and dry-meal mixtures, as much as 30 percent water is added to produce mixtures that will pass through the ricer or that can be fed easily by hand or with a spoon. The amount of water required will depend on the moisture content of the meat and on the percentage of dry meal in the diet.

Blower Feeds. --Blower-mixed feeds are excellent for feeding meat and dry meals in ponds. The food is prepared by rough-mixing the meat and dry meal and then putting it through a centrifugal blower similar to those used for room ventilation (Tunison 1945). The resulting product has the appearance of moist sawdust and is fed by scattering small quantities over the water so that the fish consume the feed as it passes through the water. Rapid feeding causes an accumulation on the pond bottom and results in loss of food for the fish.

This food is excellent for trout from the time they are placed in the ponds until they become 3 inches in length. Because the particles are fine, blower feed is not efficiently utilized by larger trout.

Cement-Mixer Food. -- Food prepared by means of a cement mixer (Tunison 1945) is excellent for fish from 3 inches in length to the size of brood stock. The food particles are larger than those of blower feed and are more completely consumed by the trout. For trout smaller than 3 inches, this type of food is less efficient than the blower feeds or bound diets.

Cement-mixer food is fed in the same manner as blower feed. Although careful feeding is necessarily slow feeding, the time is well spent because the production of trout is the reason for the existence of a trout hatchery, and any means of increasing production and improving the product cannot be regarded as waste of time.

Pelleted Food. --Several years ago a pelleted type of trout food became available. The preparation of this trout food is similar to the established method of manufacturing chick and rabbit pellets. Since the appearance of the original pellet, literally dozens of different types have appeared on the market. The pelleted food has captured the imagination of both the fish culturist and the researcher, and a tremendous amount of work has been spent in refining the methods of its manufacture and use.

Among the advantages of pellets are these points: pellets are readily stored and easily fed to the fish, and the tedious and time-consuming task of mixing diets is eliminated. A number of experiments have shown that pellets, for the most part, are about half again as efficient in conversion of food to fish flesh as the standard types of diets. The main restriction on the use of pellets is that they cannot be fed for long periods without feeding meat at least once a week, and preferably twice, if serious mortalities are to be avoided. Of course, this means that the pellets are deficient in some unknown factors. However, if meat is fed at least once a week, and pellets are fed the remaining days, there is still a saving of time and labor.

Pellets are not recommended for trout less than 3 inches in length. For trout between 3 and 4 inches in length, a small pellet (3/32 inch in diameter by 1/8 inch in length) is used; and for fish longer than 4 inches, a larger pellet (1/8 inch in diameter by 1/4 inch in length) is used. A still larger pellet (about the size of rabbit pellets) is fed to brood fish.

When pellets are used, there is almost no loss of nutrients to the water and the food consumption is nearly 100 percent. The meat feedings are usually equal parts of beef liver and beef spleen bound with 2 percent salt. For economy, ease of feeding, and efficiency of food preparation, the pellets-and-meat feeding program is the best yet devised. Perhaps some day a nutritionally complete pellet will be available and the feeding of meat can be eliminated. At present, this is impossible, and pellets should never be used without meat feedings at least once a week.

Amount To Feed

One of the most common errors in hatcheries is either to overfeed or to underfeed the trout. If overfeeding is wasteful in terms of unconsumed food, underfeeding is just as wasteful in terms of lost production. To obtain maximum production during a growing season, careful attention must be given, on a daily basis, to the amount of food the fish are receiving. In the tables that have been prepared as guides to the feeding of trout (Deuel et al. 1952), the amount of food to feed is expressed in terms of percentage of the body weight of the fish. The suggested amount is dependent on the size of the trout and the temperature of the water. For a given fish size, the amount to feed increases with increasing water temperature; and for a given water temperature, the amount to feed decreases with increasing fish size.

It is important to realize that both of these variables (water temperature and fish size) are determining factors of the

metabolic rate of trout. With an increase in water temperature, there is an increase in metabolism, indicating an increased growth potential, and more food is needed. As the fish increase in size, the metabolic rate decreases, indicating a decrease in growth potential, and less food is needed. In a feeding program, full advantage should be taken of the growth potential of the animal, thus ensuring the maximum efficiency of the diets.

It is emphasized that the feeding tables are called a guide for determining the amount of food to give trout. It is true that if the amounts shown in the feeding tables are fed, fair to excellent results will be obtained. However, there are situations in which these amounts should be increased or reduced, as determined by the specific conditions of the hatchery. When the water begins to warm in the spring, the fish indicate--by their activity and by the zeal with which they feed--an acceleration in metabolism. At this time of the year, it is possible to feed in excess of the amounts in the tables and obtain excellent conversions and an increase in the gain in weight of the fish. Experiments and experience have shown that at this time as much as twice the amounts listed in the tables can be fed, with resulting conversions of 2.5 to 3.0 pounds of food per pound of fish produced. Taking advantage of such situations increases the production of a hatchery and results in a more efficient production of fish flesh. By the same reasoning, as the temperature starts to fall, there is an apparent depressive effect on metabolism and feeding less than the amounts in the tables will result in maximum efficiency of conversion.

It is apparent that the best measure of overfeeding or underfeeding is not the amount of food that is fed, but rather the conversion factor that is obtained. A fish culturist will soon learn the "water habits" of his station; and if he gives attention to the important details of temperature and fish size, he will secure a greater efficiency than if he simply follows the guidance of the tables.

The tables of Deuel and his coauthors (1952) have been established for standard types of diets (bound, blower, and cement-mixer) in trout hatcheries. The amounts in the tables should be reduced by 40 percent when feeding pellets: if the amount of standard diet to be fed is 100 pounds, only 60 pounds of pellets should be fed. This, in itself, is an excellent indication of the efficiency of pellets. Experiments with pellets at this level have yielded conversions as low as 1.9 pounds of food per pound of fish produced, whereas conversions with standard diets at full level have been between 2.5 and 3.0.

The feeding tables group the fish by numbers per pound. This results in a large range of trout within each size group, but it is about as small a range as is practical for this type of table. It is necessary to use judgment in determining the amount to feed for a specific size of fish. If, at a water temperature of 47° F., 7.0 percent is the amount to be fed trout in the group running 2,542 to 304 per pound and 6.0 percent is the amount for trout in the group running 304 to 88 per pound, it would be poor judgment to feed 7.0 percent of their body weight to trout running 298 per pound, as 6 more fish per pound would place them at the 6.0 percent level. The fish culturist should ascertain the range position of his trout and feed them accordingly. A level of perhaps 6.5 percent would be a compromise in the foregoing example. The final decision must be based upon trout size and the water-temperature data.

The conversion factor will serve as a check; it will permit adjustments for the present growing season and establish a firm basis for judgments in future seasons.

Frequency of Feeding

There is actually no sound basis for establishing the number of feedings per day that the trout should receive. The general belief is that trout starting to feed should be fed small amounts at hourly intervals throughout the 8-hour day.

After the fish become 1.0 to 1.5 inches in length, the number of daily feedings is reduced to 4; and this is continued as long as the trout remain in troughs. Trout in

ponds are fed 3 times a day until they are 5 inches in length (growth which generally requires the first summer months of their life). Larger trout are fed twice daily until stocked. Brood fish are usually fed once a day.

The foregoing schedule is neither absolute nor ideal, but the Cortland workers believe that it is a satisfactory minimum schedule. The efficiency of feeding frequencies cannot be measured solely by growth and conversion, as uniformity of fish size, stunting of individuals, and many other phases enter the picture and are most difficult to evaluate.

As in any husbandry (human included), the fish should be fed 7 days a week.

Food Preparation and Feeding Practices

Even the perfect diet--which is yet to be found--will not produce perfect results if the feeding methods are in error. Several factors must be considered in carrying out a nutritional program in trout hatcheries.

Quality of Products. --The products fed to trout must be of good quality.

The meat products should be preserved by freezing until they are ground, and only the required amount should be ground at one time. Meats that have been allowed to stand for extended intervals are as unfit for trout as they would be for human consumption. A good standard practice is to grind sufficient meat for no more than 2 days' requirements and to keep this meat chilled until used. Excess fat should be trimmed, or--if the product is to be fed to small fish--all fat should be removed.

The dry meals that are used for trout should be finely ground and should be of the best grade available for animal feeding. It is unwise to use meals that are too coarse for the smaller trout. To avoid extensive storage, it is advisable to purchase dry meals for only 6 months' requirements. Dry meals should be stored in a cool, dry area--and very few hatcheries have the proper facilities for long periods of dry-meal storage. Under extremely adverse conditions, the storage period should be reduced.

Any food that has a rancid odor or shows other indications of spoilage must be thrown away: it should not be fed to any trout in the hatchery. The practice of feeding old, rancid foods and trimmings from meat products to the brood stock is nutritionally unsound, as the brood fish are the source of future generations and must have the best food obtainable. The quality of any animal or reproductive organism can be only as high as the quality of the food that is eaten. "We are what we eat" is certainly one of the truest statements ever made.

Food Preparation. -- Food preparation is one of the most important operations of a hatchery program. The best ingredients obtainable are useless without the proper preparation for feeding.

Meat should be ground to the proper texture for the size of the fish to be fed; it should be passed through the grinder several times if necessary. Additional water, if necessary, should be added slowly, and the food should be tested frequently during the mixing process to ensure proper consistency. An excessive amount of water lessens the bind of the diet and will cause loss of nutrients during feeding.

Meat and dry-meal mixtures should be prepared for not more than 2 days' requirements at any one time and should be refrigerated between feedings (40° F.). Because of the action of one ingredient upon another, mixed diets often deteriorate after standing for longer periods. This can be avoided by frequent preparation of the diet.

Feeding Fish. -- Care should be taken when feeding fish. Feeding is a slow process, as the fish should be fed at a rate that will allow them to consume the food as it falls through the water. The food should be broadly scattered so that all fish will have an opportunity to obtain it.

Feeding should take priority over all other husbandry operations and should be a definite routine. The daily running of a hatchery should allow sufficient time and labor to do the best possible job of feeding the fish.

Storage of Prepared Foods. --Mixed foods should be refrigerated between feedings. It is poor practice to take a day's allotment of food to a raceway or pond and leave it in the sun between feedings for the 8-hour day. Although it requires extra work to return the food to the refrigeration unit after each feeding, this must be done if the quality of the food is to be preserved.

Pellets need not be refrigerated, but all mixed or meat diets must be.

Temperature and Feedings. --Changes in water temperature from morning until evening should be considered in feeding trout. The heaviest feeding should be given during the period of the day when the water temperature is rising; the lightest, when the water temperature is falling. In most hatcheries the heaviest feeding can be given in the morning. In many hatcheries using mountain water, however, the water temperature will drop until 10 or 11 o'clock in the morning, even in summer's heat. The fish in these hatcheries should be fed more heavily in the afternoon, when the temperature is rising. Attention to details such as these can have a beneficial effect on the conversion of food to fish flesh.

Many fish culturists have adopted the rule, "Feed heavy in the morning, as the fish have starved overnight." This rule will apply in hatcheries where changes in water temperature are not a factor.

Daily as well as seasonal changes in water temperature accelerate or depress the metabolic rate. Fish feed best during periods of accelerated metabolism. The difference between satisfactory and excellent conversions of food to fish flesh depends on the attention that is given to the small refinements of fish feeding.

Accuracy of Food Weights. --Trout nutrition suffers if the ingredients of the diet are inaccurately weighed, or if an improper amount of food is fed.

An accurate scale must be available for weighing both the ingredients and the prepared diets. The degree of accuracy in weighing should be higher than 90 percent. An error of 10 percent in weighing 100 pounds of diet would mean 10 pounds of either overfeeding or underfeeding. The food, once allotted on the basis of feeding tables used with judgment, must be weighed accurately. Many conversions will be improved if only this refinement is added to the feeding program.

Each pond of trout should have its food allotted and weighed in separate containers to ensure uniformity of feeding throughout the hatchery.

Special Feeding Conditions

This article cannot cover all of the factors that may affect the feeding practices of a hatchery. Although factors such as overcrowding of the fish, unsanitary ponds, variation in fish size, disease, and disease control will affect the nutrition of trout, in this article it is presupposed that near-ideal conditions prevail for putting into effect a sound feeding program. However, because insufficient water flow and low oxygen content in the water are often unavoidable in hatcheries, these situations will be discussed.

Insufficient Water Flow. --The products of metabolism increase when trout are growing at a rapid rate and their over-all metabolism is at a peak. A fish disposes of the undesirable products of metabolism by eliminating them in the water in which he swims. Water flow and water volume dilute the products to a nontoxic level. If the products are not diluted--ponds and troughs in series are particularly prone to this--the growth rate of the trout will be reduced, the mortality will be increased, and the amount of food that is fed must be reduced.

The dilution of metabolic products is dependent upon both the flow of water into the pond and the volume of water maintained in the pond. If the water flow cannot be increased, the volume of water in the pond can be reduced to increase the number of water changes per hour through the unit and thus dilute the products of metabolism. During periods of low water flow, a reduction of the water level in the pond will often permit continuation of the optimum feeding program. Flow of water is not the limiting factor. Insufficient

water changes per hour (water flow versus pond volume) in the unit cause an accumulation of metabolic products, and this, in turn, limits production.

Low Oxygen Content in Water.--Many hatcheries suffer from a low content of oxygen in the water during the summer. The low oxygen content is often caused by a combination of insufficient water flow, warmer water, and overcrowding of the fish.

At times, however, the oxygen content is reduced by other causes. This situation frequently occurs in dirt ponds having a fairly large amount of aquatic vegetation. Often mortality occurs at night, about 4 hours after a feeding: the period of maximum use of oxygen by trout has been concurrent with the period during which plants withdraw oxygen instead of supplying it, with the result that emergency treatment--recirculating the water with a pump to increase the oxygen content of the water--is necessary.

Proper feeding methods will carry the trout through this critical period. Feedings should be scheduled so that photosynthesis is maintained during the hours of maximum demand for oxygen. Partial rations should be used: the reduction in mortalities will more than offset decrease in growth.

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The application of DDT spray to control spruce budworm in the Yellowstone River drainage, and the subsequent reduction in invertebrate populations in sprayed streams, may have been responsible for the unusual mortalities among whitefish, brown trout, and suckers in that area during the fall of 1955.

YEAR'S TRAVEL BY TAGGED RAINBOW TROUT

The record of a tagged rainbow trout that moved more than 200 miles in a little more than a year has been reported from Philipsburg, Montana, by John C. Spindler and Jack E. Bailey, fishery biologists in the employ of the Montana Department of Fish and Game.

On August 16, 1954, a 10-inch rainbow trout, jaw-tagged, was released in the Big Hole River near Ralston, Montana. The trout was of a fall-spawning strain and was about 1.5 years old at the time of planting.

On September 25, 1955, the fish was taken by an angler in the Missouri River near Townsend, Montana, approximately 210 stream miles below the point of release. During its life in the stream, the trout had grown about three-quarters of an inch in total length.

THE EFFECTS OF TAGS ON THE RATE OF GROWTH AND CONDITION OF SEVERAL SPECIES OF COLD-WATER FISH IN WYOMING

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PLACING A TAG OR SOME DEVICE ON A FISH for the sake of recognizing that particular individual among its many thousands of relatives is much like the naming of a newborn infant. However, the effects on the physical well-being of the individuals concerned are somewhat different. The pros and cons of the various types of fish tags available have been expounded, but the ideal tag for all purposes on all species of fish apparently has not been developed. Such a device or system of identifying individual fish is much in demand and is receiving much consideration. This summary of the effects of tags on trout and whitefish is made simply to add further reason for continuing the search for a better system of identifying individuals in a fish population.

Observations made by the Fisheries Management Crew that has been working in northeastern Wyoming have indicated, after a study of several years, that more tagged than untagged fish were retarded in their growth and conditioning during the time in question. Finclipping, as a means of marking a lot of fish, also seems to show an effect on the condition of fish, although to a lesser extent than tagging. The difference was declared to be nil by Cooper (1951). It appears that, though insignificant in individual comparisons, the consistency with which the difference held during this study may prove significant in the final analysis. Cooper's work was done with hatchery-stocked fish, whereas the following compilation of data is based entirely on wild stock.

Upon comparing the length and weight measurements of fish at the time of tagging with similar measurements taken after the fish had been tagged for a period of time, it was found that many of the fish failed to show a gain in either length or weight or both, and that if a gain was recorded, it was below normal expectations. The most common decrease was noted in the weight measurements. In addition to collecting these facts, Fisheries Management Crew members have periodically contacted anglers for further information. Data were also abstracted from the information secured during the operation of several other projects, but no data were secured from an experiment set up for the specific purpose of answering some of the questions about tagging. Thus, much of the desirable information necessary for a comparative analysis was not taken on projects during which tagging was done. Enough complete information was available and has been compiled here, however, to give a fair picture of what happens when a fish is burdened with some physical identification mark.

Of the several thousand fish that were tagged in this management area, a total of 216 individual fish were recovered 1 to 3 times each by the management crew. Thus, of 274 recoveries that were made on the 216 fish, 30 (13.9 percent) were seconds and 14 (6.48 percent) showed up the third time. This same lot of 216

fish was composed of 115 (53.24 percent) brown trout, 95 (44 percent) rainbow trout, 4 (1.85 percent) whitefish, and 2 (0.926 percent) cutthroat trout, taken from 5 different bodies of water (1 lake and 4 streams). Three different types of tags, attached in 5 different places on the fish, were represented. Only 2 types of tags, the band and the strap tag, showed prominently in the sample. These 2 types, applied to the jaw of the fish, represented 80 percent of the recoveries. The strap tag in the gill cover of the fish represented about 14 percent. Strap tags in the dorsal fin or at the base of the caudal fin made up 2.3 percent of the lot. Two Peterson tags, 1 through the back of the fish and 1 through the caudal peduncle, were also recovered in the lot.

Of the jaw-tagged fish, more than 95 percent had a band-type tag. This type of tag, applied around the mandible (lower jaw) of the fish, has been the most often used system of the alternatives for marking thus far developed for our work. Unfortunately, there were not enough of the various types of tags among the recoveries to make a true evaluation; likewise, the positions and points of attachment on the fish were not represented in great enough numbers to evaluate the desirability of each. Both the type of tag and the place of attachment have a very important bearing on the well-being of the fish concerned. The effect of clipping a fin or tagging a fish may certainly influence the attitude of fish toward feeding and, in turn, toward being available for capture. Cooper (1951) found the return of fin-clipped hatchery-stocked fish to the anglers' creels to be considerably greater than the return of tagged fish of the same stock, planted in equal abundance in identical conditions.

Table 1 shows the average growth in inches for the various species of fish that have been tagged and recovered since 1951 in the various bodies of water. The sizes of these fish ranged from 6.7 to 23 inches at time of tagging. The size classes of the fish, in the order of their abundance in recoveries, was as follows:

Size of fish (Inches)	Percent
10 to 12	23.6
8 to 10	18.5
16 and over	17.1
12 to 14	15.3
6 to 8	14.4
14 to 16	11.1

From this size distribution it is plain that more than 85 percent of the marked and recovered fish were more than 8 inches long at time of tagging. From table 2 it is seen that practically all of these fish were in their third year of life or older.

On comparing the average growth or increment of the tagged fish for any period of time (table 1) with the average increment for untagged fish from the same body of water for a like period of time (table 2), it is apparent that the fish do not maintain their natural growth rate. The effects on growth are more apparent than the effects on condition. Still more obvious than the foregoing comparison is the tendency for most fish to lose weight or to fail to gain in some stage of their growing period after tagging. For most fish, this static period occurs immediately after tagging and is followed by what appears to be a retarded growth rate. Other fish, which have previously had a good growth rate, have this rate gradually decline after tagging until it is very retarded. Those fish which start very slowly seem to progress at a very consistent rate even though retarded.

Sufficient information was taken from 149 of the 216 tagged fish recaptured to be desirable for use in comparing their condition at the time of tagging with their condition at the time of recapture. These 149 fish were recaptured 1 to 3 times each, for a total of 218 recaptures. The coefficient of condition--

$$C = 22.038 \times \frac{W \times 10}{L^3},$$

W being the weight of the fish in grams, and L being the total length in inches--was calculated for each fish at the time of tagging and at the time of each recapture (tables 1 and 3).

TABLE 1.--Average growth in inches during elapsed tagged time

Body of water	Species	0 - 1.75 months		2 - 3.75 months		4 - 5.75 months		6 - 8.75 months		9 - 11.75 months	
		Number	Average diff.	Number	Average diff.	Number	Average diff.	Number	Average diff.	Number	Average diff.
Clear Creek, Johnson County---	Brown---------	0	-------	36	+0.7554	5	+1.25	36	+0.551	12	+1.287
	Rainbow-------	0	-------	29	+.37	2	+.6875	21	+.8675	4	+1.265
Tongue River, Sheridan County	Brown---------	1	0.0	4	+.281	3	+.225	7	+.0486	2	+.65
	Rainbow-------	1	+.25	0	-------	0	-------	0	-------	0	-------
	Whitefish-----	0	-------	1	-.375	2	+.125	0	-------	0	-------
Sand Creek, Crook County------	Brown---------	0	-------	0	-------	0	-------	0	-------	3	-.02
Lake DeSmet, Johnson County---	Rainbow-------	7	-.25	0	-------	0	-------	0	-------	27	+1.871
	Cutthroat-----	0	-------	0	-------	0	-------	0	-------	0	-------
North Fork, Shoshone River Trout Creek, Park County.	Cutthroat-----	0	-------	0	-------	0	-------	0	-------	0	-------
						SUMMARY					
	Brown---------	1	0.0	40	+.708	8	+.8656	43	+.4693	17	+.9884
	Rainbow-------	8	-.1875	29	+.37	2	+.6875	21	+.8675	31	+1.7926
	Whitefish-----	0	-------	1	-.375	2	+.125	0	-------	0	-------
	Cutthroat-----	0	-------	0	-------	0	-------	0	-------	0	-------
	All species---	9	-.1667	70	+.7125	12	+.7125	64	+.60	48	+1.5078

Body of water	Species	12 - 17.75 months		18 - 23.75 months		24 - 25 months		36 months		Total
		Number	Average diff.	Number	Average diff.	Number	Average diff.	Number	Average diff.	
Clear Creek, Johnson County---	Brown---------	16	+1.4935	3	+3.1875	0	-------	0	-------	108
	Rainbow-------	6	+1.7542	1	+2.57	0	-------	0	-------	63
Tongue River, Sheridan County	Brown---------	2	+1.40	1	+3.235	0	-------	0	-------	20
	Rainbow-------	0	-------	0	-------	0	-------	0	-------	1
	Whitefish-----	0	-------	1	+.65	0	-------	0	-------	4
Sand Creek, Crook County------	Brown---------	18	+.641	1	+1.12	5	+1.461	4	+.4525	31
Lake DeSmet, Johnson County---	Rainbow-------	7	+2.0982	4	+.9375	0	-------	0	-------	38
	Cutthroat-----	1	+1.4375	0	-------	0	-------	0	-------	1
North Fork, Shoshone River, Trout Creek, Park County.	Cutthroat-----	0	-------	1	+.025	0	-------	0	-------	1
						SUMMARY				
	Brown---------	36	+1.0575	5	+2.7835	5	+1.461	4	+.4525	
	Rainbow-------	13	+1.9394	5	+1.264	0	-------	0	-------	
	Whitefish-----	0	-------	1	+.65	0	-------	0	-------	
	Cutthroat-----	1	+1.4375	1	+.025	0	-------	0	-------	
	All species---	50	+1.2944	12	+1.7427	5	+1.461	4	+.4525	

TABLE 2.--Age and growth data, collected by Management Crew No. 3, presented by species, age, and waters inhabited

	I	II [1]	III	IV	V	VI	VII
Tongue River							
Brown							
Length [2]	4.3	7.4	10.8	13.7	--------	--------	--------
Increment	3.1	[3] 3.4	2.9	--------	--------	--------	---
Whitefish							
Length [4]	3.87	8.05	10.255	11.35	12.45	13.30	--------
Increment	4.18	2.2	1.1	1.1	.85	--------	---
Clear Creek							
Brown							
Length [5]	6.4	9.1	12.7	13.3	--------	--------	--------
Increment	2.7	3.6	.6	--------	--------	--------	---
Rainbow							
Length [5]	5.8	9.3	12.0	--------	--------	--------	--------
Increment	3.5	2.7	--------	--------	--------	--------	---
Sand Creek							
Brown							
Length [2]	4.6	7.5	10.75	11.8	12.6	13.4	14.0
Increment	2.9	3.25	1.05	.8	.8	.6	---

[1] Practically all fish tagged in this age class or older.
[2] Length frequency.
[3] Increments at this stage are indications of the rate at which most tagged fish would be growing if left unmolested in the wild.
[4] Calculated.
[5] Average length at capture time by age.

From these computations it was shown that in 63.76 percent of the instances prior to recapture, fish had lost condition at an average rate of 5.1275 C units. This is considerable when it is noted that the average coefficient of condition for this lot of fish was 38.4314 at time of tagging. The remaining 36.24 percent of the lot, which showed gain in condition, did so at the rate of 3.387 C units.

Table 4 shows the comparative effects of tags on fish condition in four bodies of water. These fish are individuals that were tagged for periods of time ranging from 2 months to 2 years, through all seasons of the year (as fish condition varies with the seasons); and all are wild stock captured and recaptured by various methods from 3 streams and 1 lake. The cross section is inclusive enough to give ample basis for comparison. Only in a few situations where the number of fish indicated is very small, is there unsound value in the comparison.

The most obvious results of the effects of tags on fish populations are shown in table 4, where the coefficient of condition at the time of tagging is compared with the condition factor at recapture.

This summary is not meant to show conclusively that all tags are detrimental to all fish which receive tags, but it does

TABLE 3.--Average change in weight (ounces) during elapsed tagged time

Body of water	Species	0 - 1.75 months		2 - 3.75 months		4 - 5.75 months		6 - 8.75 months		9 - 11.75 months	
		Number	Average diff.	Number	Average diff.	Number	Average diff.	Number	Average diff.	Number	Average diff.
Clear Creek, Johnson County.	Brown------	0	-------	36	+0.7842	0	-------	15	+2.18	13	+2.2531
	Rainbow----	0	-------	29	+.2879	0	-------	13	+1.7554	4	+4.12
Tongue River, Sheridan County.	Brown------	0	-------	3	-.1167	0	-------	0	-------	1	+3.6
Sand Creek, Crook County.	Brown------	0	-------	0	-------	0	-------	0	-------	0	-------
Lake DeSmet, Johnson County.	Rainbow----	5	-4.4	0	-------	0	-------	0	-------	25	+9.36
	Cutthroat--	0	-------	0	-------	0	-------	0	-------	0	-------
	SUMMARY										
	Brown------	0	-------	39	+.7149	0	-------	15	+2.18	14	+2.3493
	Rainbow----	5	-4.4	29	+.2879	0	-------	13	+1.7554	28	+8.95
	Cutthroat--	0	-------	0	-------	0	-------	0	-------	0	-------
	All species	5	-4.4	68	+.5328	0	-------	28	+1.9829	42	+6.7498

Body of water	Species	12 - 17.75 months		18 - 23.75 months		24 - 35.75 months		36 months and over	
		Number	Average diff.	Number	Average diff.	Number	Average diff.	Number	Average diff.
Clear Creek, Johnson County.	Brown------	15	+3.0387	2	+9.125	0	-------	0	-------
	Rainbow----	4	+3.13	1	+9.0	0	-------	0	-------
Tongue River, Sheridan County.	Brown------	2	+1.375	1	-3.9	0	-------	0	-------
Sand Creek, Crook County.	Brown------	2	+.23	0	-------	1	-2.34	1	-1.28
Lake DeSmet, Johnson County.	Rainbow	5	+5.8	4	-2.0	0	-------	0	-------
	Cutthroat--	1	+9.0	0	-------	0	-------	0	-------
	SUMMARY								
	Brown------	19	+2.5679	3	+4.7833	1	-2.34	1	-1.28
	Rainbow----	9	+4.6133	5	+.20	0	-------	0	-------
	Cutthroat--	1	+9.0	0	-------	0	-------	0	-------
	All species	29	+3.4245	8	+1.9188	1	-2.34	1	-1.28

TABLE 4.--Comparison of coefficients of condition and changes of coefficients for 149 trout recaptured a total of 218 times after tagging

	Increase C	Decrease C	Average C at tagging	Average C at recapture
Brown trout				
Tongue River				
Number	6	3	9	9
Average change in C	4.0867	4.9067	32.2022	33.2911
Sand Creek				
Number	------	1	1	1
Average change in C	------	.1000	39.5600	39.4600
Clear Creek				
Number	40	64	104	104
Average change in C	2.7898	5.4591	38.5425	36.2561
Total brown trout				
Number	46	68	114	114
Average change in C	2.9589	5.3559	38.0509	36.0501
Rainbow trout				
Clear Creek				
Number	15	47	62	62
Average change in C	4.5207	5.4885	40.7533	37.0290
Lake DeSmet				
Number	17	24	41	41
Average change in C	3.7406	3.7733	37.0322	36.3744
Total rainbow trout				
Number	32	71	103	103
Average change in C	4.1063	4.9087	38.8764	36.7684
Cutthroat trout				
Lake DeSmet				
Number	1	------	1	1
Average change in C	.0640	------	35.9660	36.0300
Total trout				
Number	79	139	218	218
Average change in C	3.3870	5.1275	38.4314	36.3894

represent some of the relative effects that should not be overlooked when the tagged fish are being utilized for other considerations. Poor condition resulting from tagging may also be a factor to be considered when studying the survival of fish.

Acknowledgment

Acknowledgment of assistance is due Fisheries Management Crew No. 3 of the Wyoming Game and Fish Commission for work in tagging, collecting data, and maintaining the records on which this summary is based. The author is now a member of this crew.

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PRELIMINARY OBSERVATIONS ON THE LOWERING OF DISSOLVED OXYGEN BY SODIUM SULFITE AND ITS EFFECTS ON CERTAIN FISHES, WITH PARTICULAR REFERENCE TO PROBLEMS IN FISH MANAGEMENT

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THE NEED FOR AN EFFECTIVE AND ECONOMICAL METHOD OF SALVAGING CERTAIN FISHES FROM LAKES AND PONDS, and thinning the populations of other species, has long been manifest in the field of fish management. Rotenone, though varyingly effective for "wiping out" fish life, has the disadvantage of (1) dooming fish to almost certain death, and (2) rendering the lake or pond unfit for fish life for several days or weeks unless followed by treatment with an oxidizing agent such as was described by Lawrence (1956). Electronic devices, effective in certain stream situations, are as yet inadequate for the desired results in lakes and ponds. Cresol, reported on by Embody (1940), Embody and others (1941), and Wilkens (1955), acts quickly in streams; and it leaves a stillwater habitat in a highly undesirable condition for an unknown period of time.

Laboratory and pond experiments in lowering dissolved oxygen content by the addition of sodium sulfite have indicated that this treatment may be a valuable means of salvaging and thinning fish populations in small ponds and in lakes that can be lowered to a few acre-feet of water. The method is apparently nontoxic, and the degree and speed of response can be controlled to desired extents. Further, the treatment renders the pond unsuitable for aquatic life for little longer than a day or so.

The procedures and results of the laboratory experiments will be described first; next, the procedures and results of the field experiment.

Laboratory Observations

All laboratory experiments, preliminary or otherwise, were conducted under certain consistent procedures as follows:

1. All aquaria were of commercial, 5-gallon capacity and dimensions.
2. The quantity of water employed in each aquarium was 12 liters.
3. The water employed was taken from municipal supply and was put into the aquaria at least 20 hours before experiments were started.
4. All experimental fish were young-of-the-year largemouth bass from a stock supply that had been maintained in a 65-gallon aquarium for several weeks prior to this experimental work. These fish were 2 to 3 inches in length.
5. Three fish were transferred to each of the experimental aquaria approximately 5 minutes before experimentation was begun.
6. Sodium sulfite, either in the form of powder or in a saturated, aqueous solution, was added to the aquaria.
7. All measurements of dissolved oxygen, including measurements that were made in the field experiment, were made

by the polarographic method of Rand and Heukelekian (1951). This method was selected because it is a simple procedure adaptable to the determination of dissolved oxygen in the presence of a reducing agent such as sulfite.

Series of preliminary experiments were first conducted to observe the reactions of the bass when various amounts of sodium sulfite were added to the water. The dosage rates were: 56 p.p.m., 112 p.p.m., 168 p.p.m., 224 p.p.m., 280 p.p.m., and 560 p.p.m. The dosage of 56 p.p.m. represented the approximate amount of sodium sulfite theoretically necessary to reduce the dissolved oxygen content to zero if no reoxygenation of the water took place. The water temperature in all 7 of the experimental aquaria was maintained between 72° and 74° F.

In all the foregoing experiments that involved dosages of 112 p.p.m. or higher, the fish came to the surface after an interval of time and began to gulp. Shortly after the fish began to gulp at the surface, they became easily catchable by hand or with a small dip net. The period of time during which the fish remained easily catchable at the surface by these means will be referred to as the period of catchability or catchability period. This period of catchability ended when the fish sank to the bottom; and if fish were to be revived, it was necessary to transfer them before they had remained on the bottom for a half minute or so. All fish transferred during the catchability period revived. It was discovered that the young bass could be subjected to the treatment at least three times within a week without exhibiting any symptoms of injury or weakness.

The duration of the catchability period, and the interval of time before its onset, varied with the dosage of sodium sulfite. At 56 p.p.m., for example, there was no catchability period; whereas at 560 p.p.m., the time before onset was usually about 15 minutes and the period of catchability lasted only about 5 minutes. At a dosage of 168 p.p.m., both the time interval and the catchability period were sometimes more than an hour.

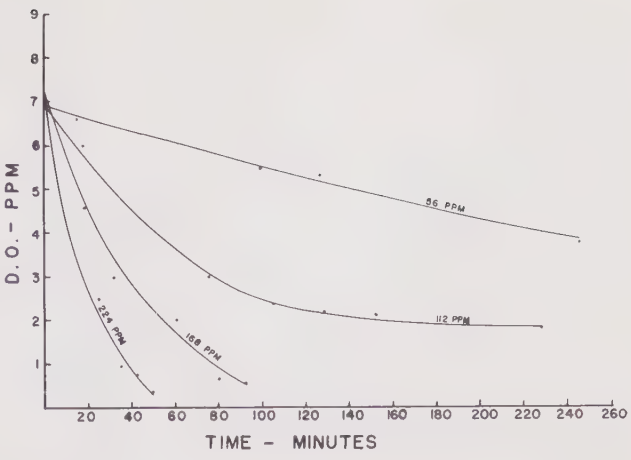

FIGURE 1.--The rates of decreases in dissolved oxygen at various dosages of sodium sulfite. (Curves have been sight-fitted.)

After these preliminary observations, a second series of experiments was conducted at 72° F. with dosages consisting of 56 p.p.m., 112 p.p.m., 168 p.p.m., and 224 p.p.m. Determinations of dissolved oxygen were made by the polarographic method during these experiments, and pH values were obtained colorimetrically.

Decreases in the amounts of dissolved oxygen at the various dosages of sodium sulfite, together with the rates of these decreases, are shown in figure 1. Measurements of pH gave a value of 7.0 for the untreated water, a rise to 8.4 at dosages of less than 224 p.p.m., and a rise to 8.6 at the 224 p.p.m. rate. Total alkalinity of the untreated water was approximately 33.

The catchability period in these experiments took place when the dissolved oxygen had decreased to approximately 1 p.p.m., and it was observed that the fish died within a few minutes at a dissolved oxygen level of less than 1 p.p.m. when they were denied access to the surface. If, on the other hand, the fish were permitted to gulp at the surface, they could survive for an hour or more at a dissolved oxygen level of approximately 0.8 p.p.m.

After these experiments with various dosages of sodium sulfite at a temperature

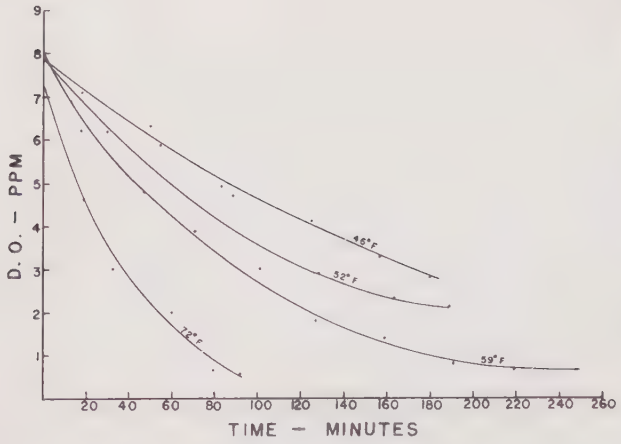

FIGURE 2.--The rates of decreases in dissolved oxygen at various temperatures, at a dosage of 168 ppm of sodium sulfite. (Curves have been sight-fitted.)

FIGURE 3.--Farm pond used in field experiment. The near (lower) end shows the dock and boat mentioned in the text. (Illustrations courtesy of the authors)

of 72° F., another series was conducted at different temperatures employing a constant dosage (168 p.p.m.) of sodium sulfite. The temperatures were 46°, 52°, and 59° F., and they were maintained by placing the aquaria on different levels in an open-type refrigerated display case. (It should be mentioned in passing that this type of refrigerated case proved to be an excellent piece of equipment for obtaining any constant temperature desired between near-freezing and room temperature.)

Polarographic measurements of dissolved oxygen were made again during these experiments, and the retarding effects of the lower temperatures on the action of the sodium sulfite have been portrayed in figure 2. It will be noted from this graph that the action of the chemical at 52° and 46° F. was not sufficiently rapid to combat the rate of re-aeration at the surface and produce a catchability threshold. On the other hand, there was a long period of catchability at 59° F.

It was also learned from these experiments that the sodium sulfite powder would lump badly and sink to the bottom at temperatures below 59° F. This occurred even when an N. F. grade of exsiccated powder--a product that dissolves almost instantly at temperatures above 58° F.--was used.

Field Observations

On October 9, 1955, a small farm pond (figure 3) on the property of the senior author at New Market, New Jersey, was treated with sodium sulfite (N. F. grade of exsiccated powder) at a dosage of approximately 168 p.p.m. This pond had been stocked 3 years previously with some adult golden shiners (Notemigonus crysoleucas) and a few dozen catfish (Ameiurus nebulosus). It had also been stocked, 2 years previously, with 26 juvenile largemouth bass. During 1954 and 1955, several thousand bait-sized golden shiners had been removed. Further, the pond had been invaded--possibly in 1953--by common sunfish (Lepomis gibbosus) and by some other undesired species, through a muskrat tunnel under the outlet screen.

The pond was 145 feet by 45 feet, with an estimated mean depth of 4 feet and a maximum depth of 6 feet.

The banks of the pond were steeply sloped except at the upper end (far end in figure 3), where there was a shallow area; and the location of the pond within 100 feet of the house provided excellent opportunity for long and close observation in experimental work.

At the time of experiment, the pond was supporting a moderate plankton bloom

and the day was characterized by bright sunlight and a fresh-to-strong wind sweeping the pond from the lower to the upper end. The surface temperature at the lower end was 63° F., and the bottom temperature was 61° F. Dissolved oxygen was approximately 6.3 p.p.m.

Treatment was begun at 12 o'clock noon by throwing 2 or 3 quarts of sodium sulfite at a time into the wash of a 7.5-horsepower outboard motor that was running at two-thirds speed on a boat fastened to the dock at the lower end of the pond (figure 3). After some 175 pounds were put into solution in this manner, 60 pounds more were put into the wash of the outboard as the boat was cruised slowly on the pond. The boat was then cruised 10 minutes more to ensure thorough mixing of the chemical.

Within 15 minutes after the application, golden shiners began to gulp at the surface and salvage operations with dip nets started. Largemouth bass, catfish, chubsuckers (Erimyzon oblongus), common suckers (Catostomus commersonni), carp, goldfish, and common sunfish began to surface some minutes later.

As it was desired that only catfish and adult golden shiners should be returned to the pond, only these, together with samples of the other species, were selected for salvage experiment. On the other hand, some of all the observed species were allowed to remain in the pond in order that their fate might be determined. Salvaged fish were first placed in containers of water and then transferred, within a matter of minutes, to five bathtubs that were connected in series with running water.

All fish salvaged revived within 5 minutes and included the following:

Species	Approximate number
Largemouth bass	17 adults, 3 "zeros"
Golden shiner	225 adults
Catfish	87 adults (?) up to 14 inches in length
Carp	1 adult
Goldfish	1 adult
Common sunfish	5 adults, 2 juveniles
Chubsucker	3 adults
Common sucker	1 juvenile

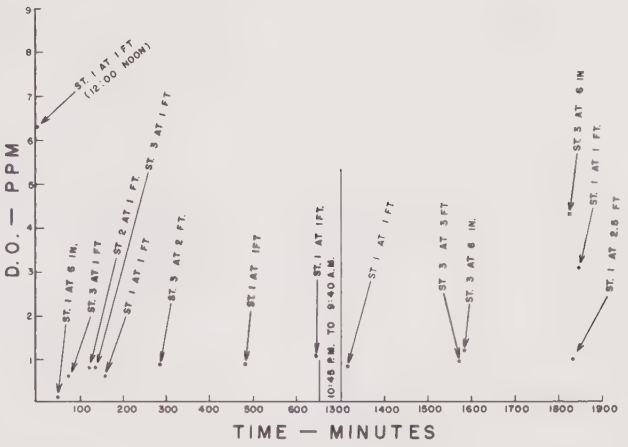
D.O. — PPM
TIME — MINUTES
ST. 1 AT 1 FT (12:00 NOON)
ST. 1 AT 6 IN.
ST. 3 AT 1 FT
ST. 2 AT 1 FT
ST. 3 AT 1 FT
ST. 1 AT 1 FT
ST. 3 AT 2 FT.
ST. 1 AT 1FT
ST. 1 AT 1FT.
10:45 P.M. TO 9:40 A.M.
ST. 1 AT 1 FT
ST. 3 AT 3 FT
ST. 3 AT 6 IN.
ST. 3 AT 6 IN.
ST. 1 AT 1 FT.
ST. 1 AT 2.5 FT

FIGURE 4.--Effects of sodium sulfite on dissolved oxygen in a farm pond (see figure 3) when applied at a rate of approximately 168 ppm. Station locations: (1) at lower end of pond, (2) middle of pond, (3) 40 feet from other end of pond.

Particular attempts were made to destroy as many sunfish as possible during this experiment, and it was discovered that thousands of "zeros" and juveniles could be scooped up in a small scap net from shore during the several hours before nightfall. Adult common sunfish seemed to be somewhat resistant to the conditions for several hours, but upwards of 400 were found, together with several "zero" bass, dead and floating at the surface of the pond the morning after the treatment.

Carp, goldfish, and suckers did not seem able to endure, for more than an hour or so, the low oxygen content portrayed in figure 4. All exhibited a characteristic behavior of skipping or "greyhounding" over the surface of the water and expiring shortly thereafter.

The behavior of largemouth bass was unique in that they did not surface near the middle of the pond but rather hid among, or under, the vegetation of the shoreline. Indeed, special efforts were necessary to detect them.

The most remarkable behavior was that of the catfish and golden shiners that were allowed to remain in the water. During

the afternoon of October 9 and until 5:15 p.m. on October 10, both these species, singly or in groups, gulped at the surface of the pond. Suddenly, at 5:15 p.m. on October 10, they disappeared from the surface. A measurement of dissolved oxygen some 45 minutes later showed a content of 4.3 p.p.m. near the surface (figure 4). All of the salvaged catfish and golden shiners were returned to the pond during the evening of October 10, and no mortality was observed at that time or later.

Comment

It seems apparent that use of sodium sulfite has considerable value as a means of salvaging fish in certain types of situations and as a simple means of observing the reaction of fishes under conditions of reduced amounts of dissolved oxygen.

It also seems obvious that the method, owing to the cost of the chemical (approximately 10 cents a pound), is not practical for large bodies of water and is limited to small ponds or to lakes that can be lowered to a few acre-feet of water.

The results of the field experiment suggest that effective salvage might be effected through the use of a somewhat lower dosage of sodium sulfite--for example, between 112 and 140 p.p.m.--particularly at higher temperatures.

The exact means by which oxygen is obtained at the surface through gulping is not understood. Samples of water that were taken from within 3 millimeters of the surface in experimental laboratory aquaria failed to show any significant difference in dissolved oxygen. It was noted, however, that all fishes with inferior mouths--that is, carp, goldfish, and suckers--in the pond experiment appeared to suffer a hundred percent mortality.

Acknowledgments

The authors wish to express their gratitude to the several persons who assisted in this work. Particular thanks are due Mr. Donald Riemer, of Rutgers University, for his assistance with the laboratory and field work and with the preparation of some of the figures that appear in the text. Special thanks are also due Mrs. James R. Westman for her assistance in the field work, and to Dr. Walter A. Maclinn and the Department of Food Technology of Rutgers University for their cooperation in making available certain laboratory facilities and equipment. The authors also wish to thank Mr. Grant F. Walton, of Rutgers University, for his assistance with the laboratory experiments; and Mr. M. S. Spulock, of New Market, New Jersey, for his assistance in the field work.

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ESTABLISHMENT OF A CONTROLLED-FLOW AREA AND CONSTRUCTION OF KING SALMON SPAWNING PENS AT MILL CREEK, CALIFORNIA

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U. S. Fish and Wildlife Service
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ACCURATE INFORMATION ON THE EARLY LIFE HISTORY OF THE KING SALMON (Oncorhynchus tshawytscha) is essential to an understanding of the causes of fluctuations in abundance. Because it is virtually impossible to obtain these data under natural conditions, a controlled-flow stream was established by the U. S. Fish and Wildlife Service in Mill Creek, at Los Molinos, California, in cooperation with the California Department of Fish and Game.

A description of the channel and its facilities is presented because it may be of interest to hatchery workers and others concerned with spawning and holding fish.

An isolated channel that leads independently from Mill Creek to the Sacramento River was selected for conducting experimental spawning and incubation studies. Spawning pens and a water-control dam were built as essential counterparts of the experimental area. Brush, silt, sand, and gravel were bulldozed from its entrance to reestablish flow into the channel. A length of corrugated metal pipe was laid in the channel and covered with earth to create a dam extending 8 feet above the top of the pipe (figure 1). A headgate (Calco Model 101) was mounted at the upstream end of the pipe for flow regulation. A platform which supported and provided access to the headgate was extended from the top of the dam to a slab of concrete surrounding the upstream end of the culvert.

Stream improvement work was done in the old channel for approximately 1,000 feet below the dam. This involved removing large rocks, loosening gravel, and freeing silt and sand. The channel was made a uniform width, and the slopes of each riffle were made constant. Two additional settling ponds were excavated below the dam.

Three spawning riffles were constructed downstream from the settling ponds for the purpose of making controlled observations of spawning and fry production. Each was 60 feet long and 10 feet wide and had a rest pool at its lower end. To insure reliable results from the experiments, basic similarity of the pens was required. The controlled-flow feature of the channel simplified the maintenance of similar water flows. As the riffles adjoined each other, physical variations were minimized (figure 2). However, the stream-bed structure and slope required standardizing.

When the channel area was suitable, three pens were constructed to enclose the riffle areas. Pen construction required strength to withstand the onslaughts of adult king salmon and size to allow unrestricted spawning of several fish simultaneously. The pens also had to be convertible into fishtight areas to contain fry and allow adequate stream flow.

Construction of the pens basically consisted of pouring a 60-foot concrete slab, 1 foot deep and 6 inches wide, in the bed

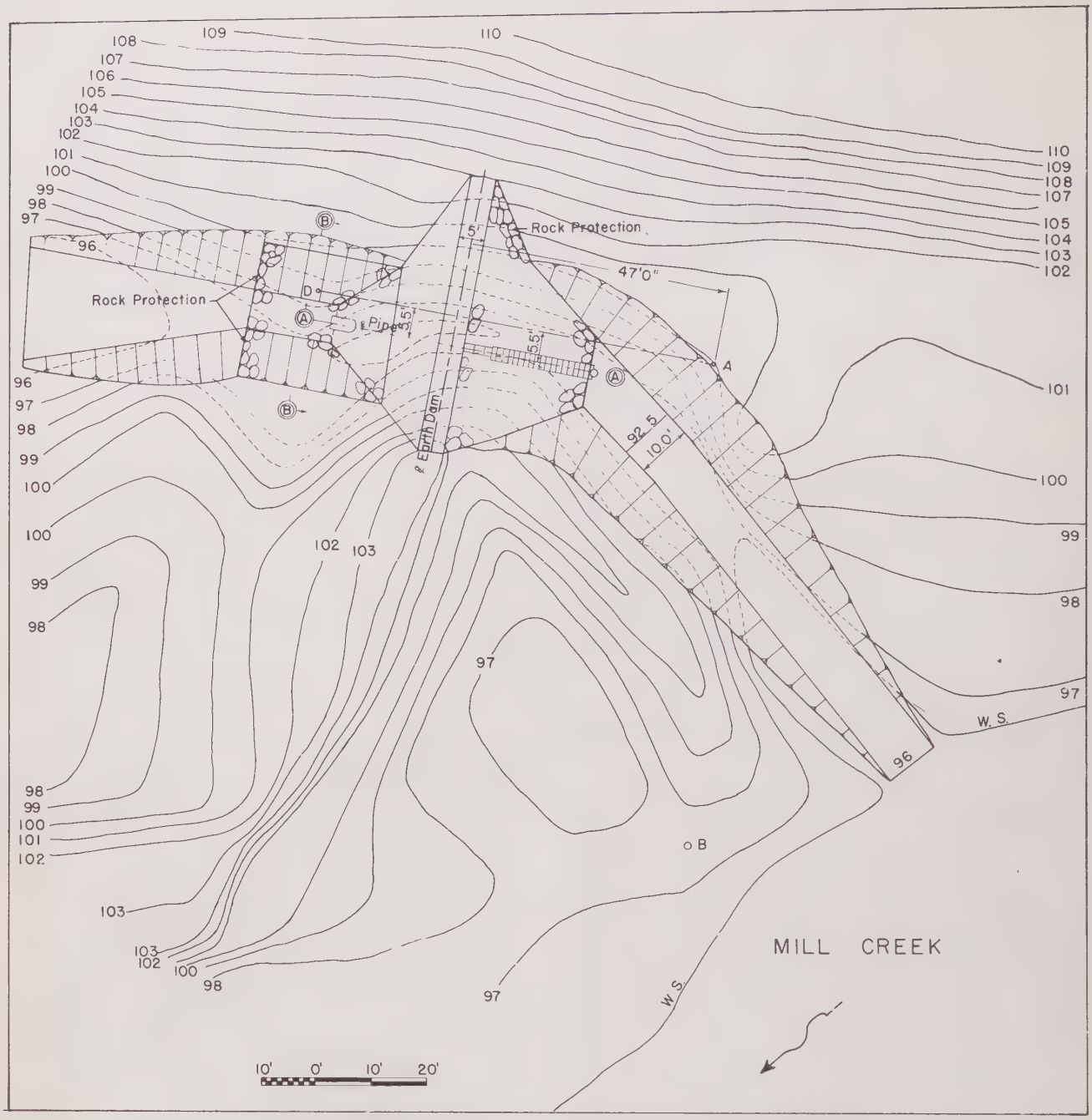

FIGURE 1.--Location plan of flow-control dam, Mill Creek, California.

of the stream so that the top of the slab was approximately level with the gravel. Before the concrete was set, pipe posts were pushed vertically into the wet cement at 8-foot intervals. Stringers were bolted to the posts; unfinished redwood pickets, 2 inches by 2 inches by 6 feet, were nailed to the stringers (figure 3). Spreaders of the same material were placed at top and bottom between the pickets.

Across the stream and between riffles, pickets 8 feet long were used. To provide a seal and to prevent undercutting where there was no concrete footing, a trench was dug down to the hardpan. The pickets were set in place against the

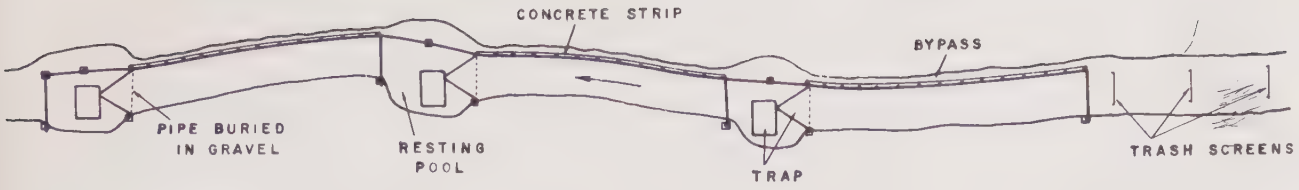

FIGURE 2.--Diagram of spawning pens in controlled-flow stream, Mill Creek, California.

hardpan; then rocks were filled in around them.

In the pen areas, gravel was loosened and silt and sand were cleared by cultivator. An additional 30 cubic yards of gravel made the spawning space comparable to a natural bed in a stream.

Trash screens were installed above the spawning pens to help keep the hardware cloth around the pens clean. The screens consisted of rectangular pieces of hardware cloth framed in angle iron and held by iron pipes set in concrete.

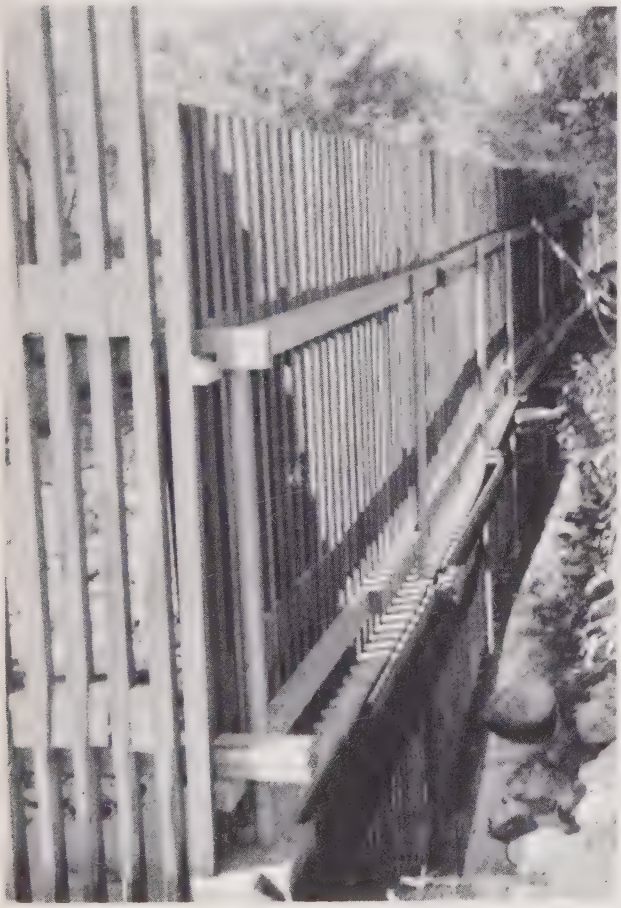

FIGURE 3.--Bypass and detail of pen construction.

Hardware cloth of 1/8-inch mesh and 36 inches wide was used to line the pens before the fry began to emerge. A seal was formed along the sides of the pens by pushing the cloth down between the concrete and the gravel. The top edge of the screen was stapled to the pickets. Hardware cloth running across the stream was creased about 6 inches from the edge. The fold of the cloth was placed flat in a trench on the upstream side of the pickets. Rocks were piled on top of this. A thorough, careful job was necessary here to prevent the current from undercutting. The top of the cloth was stapled in place.

Need for screens around the rest pools was obviated by the presence of traps in the pools at the time of fry emergence.

Picket stock was used to frame the pots of the traps. These were 6 by 8 by 3 feet high, with plywood top and bottom and the sides covered with hardware cloth. The top was hinged to permit the removal of trapped fry. A V-shaped throat section guided the fry into the trap. It was framed with 2 x 4's, the sides and bottom being covered with hardware cloth. Fry could not pass under the funnel, as the wire cloth was tucked under an iron pipe buried in the stream-bed. Each side was made fishtight.

Comments

The Mill Creek control-flow stream described has proved satisfactory for conducting king salmon spawning and egg-incubation experiments. Fry emerged

from 14.2 percent of the eggs spawned naturally in the 3 pens in the fall of 1954, the first year the spawning pens were used.

Though the production from natural spawning was not unsatisfactory, it is our opinion that improvements can be made by slight modifications of the pens. Some severe mortality took place after the hardware cloth was installed and just before emergence of fry. Algae clogging the screens slowed the current and caused excessive sand deposits in the pens. This condition will be improved by increasing the mesh size to 6 per inch. Also, the flow through the pens will be increased by widening the screen area at the head of each pen. The flow in the control stream is regulated at 10 to 12 cubic feet per second during spawning and reduced to 3 or 4 cubic feet per second during egg incubation. Reduced flow lessens sand deposits on the experimental areas by causing more settling in the pools but has little effect on silt.

At the University of Oklahoma, special studies are being made on the growth and survival problems of the channel catfish, a game and a commercial fish, in ponds.

RICE AND FISH CROPS IN ARKANSAS

More and more acres in Arkansas are being used to produce alternating crops of rice and fish. It has been estimated that 50,000 of approximately 1,000,000 acres of the State's rice lands are now being used for commercial fish production.

Reservoirs are an important tool in the production of each of these crops. In the rice-growing areas, the soil structure is such that little or none of the surface water will percolate to the substrata. Thus, to save this valuable water, dirt levees are thrown up to form reservoirs. These are used to flood the fields and to hold fish.

The cycle of rice and fish production is begun with the planting of rice. Water is pumped over the fields of growing rice and then is drawn off just before harvest time. After the rice harvest, the fields are flooded again and this time are planted with the desired fish species, such as carp, buffalo, and channel catfish. At the end of 2 years, the fields are drained again and the fish crop is harvested. The marketable fish are sold, brood stock and surplus fish are returned to the reservoirs, and any undesirable species are destroyed.

Though buffalo and channel catfish are considered difficult to propagate in controlled waters, the job is being done on a hit-or-miss basis. The farmers who have been successful in producing fingerlings of these two species have a most profitable enterprise. It is reported that buffalo fingerlings, 3 to 6 inches in length, are being sold for $50 to $75 per thousand. A rice grower who produced 700 pounds of buffalo per acre over a 2-year period recently sold his marketable fish and a considerable quantity of brood stock for only slightly less than he had received for his previous rice crop. To the price received for the fish could be added, of course, the value of stored water plus fertilizer--fertilizer the water is believed to return to the soil at the rate of approximately $20 per acre per year.

Game species produced by the rice growers are kept for sport fishing. An increasing number of reservoirs are being leased to sportfishing groups or opened to the public on a pay-to-fish basis.

URETHANE AS A CARCINOGEN

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IN A RECENT ARTICLE, urethane was described as a particularly good agent for producing lung tumors in mice (Warburg 1956). Because this substance is widely used as an anesthetic for fish, we checked the literature on its carcinogenic qualities with some concern. That this concern was justified is indicated by the summary that follows.

In an experiment involving X-ray treatments of anesthetized mice, an exceptionally high incidence of lung tumors resulted (Nettleship et al. 1943). This was shown to be caused by the intraperitoneal injection of the anesthetic, urethane. Subsequently, it was reported that a single injection of urethane will induce lung tumors (Henshaw and Meyer 1944), and that they develop whether the carcinogen is administered by injection, in the drinking water (Henshaw and Meyer 1945), by nasal instillation (Selbie and Thackray 1948), or from painting on the skin (Cowen 1950). The tumors also develop in treated rats and rabbits.

The question of immediate importance concerns the possible danger to fishery workers who have been in extended contact with urethane. To our knowledge, there is no report of humans with lung tumors

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Inquiry regarding this problem has been made of Dr. W. C. R. Hueter of the National Cancer Institute, Environmental Cancer Section, Bethesda 14, Maryland. At this time, there is no medical evidence that indicates that contact with urethane is carcinogenic to man. Evidence accumulated on other animals, however, indicates that there is a possibility that humans might get cancer from the use of urethane. It is suggested, therefore, that anyone who works with urethane should do so with the utmost caution. Rubber gloves should be worn to protect the hands, respirators should be used when the powder is being weighed for the preparation of solutions, and some precaution should be taken to protect the body from water that is splashed by fishes.

Dr. Hueter is very much interested in the method used in anesthetizing fish with urethane. He would be glad to receive any available information regarding development of tumors or similar anomalies in fish that had previously been dipped in urethane.

Possibly consideration should be given to use of an alternative, such as M.S. 222, tricaine methanesulfonate. R. T. Webb, Jr., fishery-management biologist stationed in the Atlanta, Georgia, office of the U. S. Fish and Wildlife Service, has reported the successful use of M.S. 222 not only as an anesthetic but also as an aid in fish distribution. Work undertaken for his thesis on the use of M.S. 222 to anesthetize pond fishes demonstrated that, in water at temperatures between 50° and 80° F., application of this chemical at the rate of 0.4 gram per gallon of water provided a satisfactory anesthetic for goldfish, golden shiners, and bluegills--though this concentration was lethal to fathead minnows. If applied at the rate of 0.1 gram per gallon, M.S. 222 could be of assistance in the distribution of such species as the bluegill. This concentration reduced the activity of the fish, but they remained upright, in a swimming position.

—The EDITORS.

resulting from urethane. However, this substance is known to cause a leucopenia in humans and consequently is used in the treatment of leukemia. Its action on the control of cell division is only poorly understood, and Cowen cautioned (1950: 340) as follows:

> It is not unreasonable to suppose that urethane is absorbed at least as readily from human as from mouse skin. Since urethane causes leucopenia in man (Moeschlin and Meili, 1947), ... it would be well for those who handle this substance frequently in the laboratory or commercially to consider it a possible risk to health and consequently take effective precautions.

The amount of urethane which a fishery worker contacts, in comparison to the amount required to produce tumors in mice, is especially pertinent. As little as 0.002 gram per mouse per week results in tumor production when this amount is painted on the skin in a 20-percent acetone solution (Cowen 1950). The skin absorption is apparently very high. On a similar basis, assuming that the average rat weighs 200 grams, a 150-pound human would require approximately 0.7 gram. In a gallon of 0.5 percent urethane, there will be 19 grams of urethane. Obviously, there is much that is not known about the absorption of urethane from a water solution. Until more is known about the potential damage, however, there is definite justification for concern for the health of an individual who has prolonged contact with such solutions.

It has also been established that though urethane is not in itself carcinogenic for mouse skin, the compound is able to induce in that tissue the initiating phases of carcinogenesis (Berenblum and Haran 1955). Thus, when other carcinogens are contacted, there is an increased chance that tumors will develop.

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The homing instinct is apparently developed to a rather high degree in cutthroat trout in Yellowstone Lake, making it necessary for biologists to consider the significance of that factor in relation to a management plan for that body of water.

UTILIZATION OF GIZZARD SHAD BY LARGEMOUTH BASS

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THE GIZZARD SHAD (Dorosoma cepedianum) is an ideal warm-water fish. It is prolific and does not compete with game fish for food. Dubets (1954) found that most of the members of a typical gizzard shad population were small enough to be eaten by largemouth bass (Micropterus salmoides). The fact that the largemouth bass does utilize the gizzard shad intensely indicates it to be highly vulnerable or particularly palatable. The latter may well be the case, as even dead shad are excellent bait for catfishes (Ictalurus lacustris and I. furcatus) and fresh-water sheepshead (Aplodinotus grunniens).

The present study is based on observations of the feeding habits of the largemouth bass in the presence of a dense gizzard shad population. The method of study, which is the same as that used by Dubets (1954), consists essentially of stunning the largemouth bass by means of electrical shock and then observing their stomach contents with a gastroscope.

All the largemouth bass examined in this study were taken from Crab Orchard Lake, a 6,500-acre artificial impoundment in Williamson County, Illinois. The principal fishes of the lake, listed in the order of their approximate abundance, are: gizzard shad, white crappie (Pomoxis annularis), carp (Cyprinus carpio), yellow bass (Morone interrupta), bluegill (Lepomis macrochirus), and largemouth bass.

Of 211 largemouth bass examined during the spring and summer of 1955, the stomachs of 104 were empty. In the 107 stomachs containing food, the occurrence of different food items was as follows:

Gizzard shad	85
Crayfish	7
Unidentified fish remains	6
Bluegill	3
Algae	2
Turtle	1
Largemouth bass	1
White crappie	1
Blackstripe topminnow, Fundulus notatus	1

Obviously, gizzard shad is the principal food of the largemouth bass of Crab Orchard Lake. This finding corroborates an earlier study by Dubets (1954), who reported that gizzard shad occurred in 63 stomachs out of 85 that contained identifiable food materials.

The rate of growth of the largemouth bass in the present study was analyzed in order to establish the welfare of fish subsisting primarily on gizzard shad. The growth rate (see table), when compared with similar data (Carlander 1950), was found to be very satisfactory. The bass were of good catchable size (total length, 15 inches) by the end of their third year.

It is apparent that largemouth bass utilize shad to a marked degree. If one of the principal aims of management of a lake is production of largemouth bass fishing, it is questionable that gizzard shad should be considered a weed species. In fact, it may be desirable to establish

A cooperative study made by the Illinois Department of Conservation, the Cooperative Fisheries Research Laboratory, and Department of Zoology of Southern Illinois University. The U. S. Fish and Wildlife Service gave permission to conduct the study on Crab Orchard Lake.

GROWTH OF 188 LARGEMOUTH BASS TAKEN FROM CRAB ORCHARD LAKE, SUMMER OF 1955

Age group	Number examined	Standard length at capture (mm.)	Average calculated standard length at each annulus						
			1	2	3	4	5	6	7
0	22	108	---	---	----	----	----	----	----
I	9	151	115	---	----	----	----	----	----
II	19	219	88	179	----	----	----	----	----
III	51	312	89	191	261	----	----	----	----
IV	29	368	92	206	275	326	----	----	----
V	33	407	86	199	277	330	373	----	----
VI	19	420	87	206	277	330	372	403	----
VII	6	432	89	203	268	319	360	386	409
Mean calculated standard length--(mm.)			92	197	272	326	368	395	409
Annual increment----------(mm.)			92	109	71	52	42	29	23
Equivalent total length-------(inches)			4.5	9.5	13.0	15.5	17.3	18.5	19.2

these shad in some lakes where they do not presently exist.

Summary

Gastroscopic analysis of the stomach contents of 211 largemouth bass taken during the spring and summer of 1955 revealed that, of the 107 stomachs containing food, 85 contained gizzard shad. Items of less frequent occurrence included crayfish, turtle, algae, and miscellaneous fishes. Analysis of the scales of this bass population subsisting primarily on gizzard shad indicated that the rate of growth of the population was above the average.

It was concluded that the gizzard shad is a desirable fish where management is directed toward production of largemouth bass.

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In 25 divisions, the unicellular Gymnodinium brevis can produce 33 million cells.

HARVEST FROM AN EXPERIMENTAL ONE-ACRE SALT-WATER POND AT BEARS BLUFF LABORATORIES, SOUTH CAROLINA

Correction: In Dr. G. Robert Lunz' paper, "Harvest From an Experimental One-Acre Salt-Water Pond at Bears Bluff Laboratories, South Carolina" (vol. 18, no. 2, pp. 92-94), the genus Palaemontes should be substituted for Penaeus in table 2.

USE OF LIGHT AIRCRAFT IN LAKE-SURVEY ACTIVITIES

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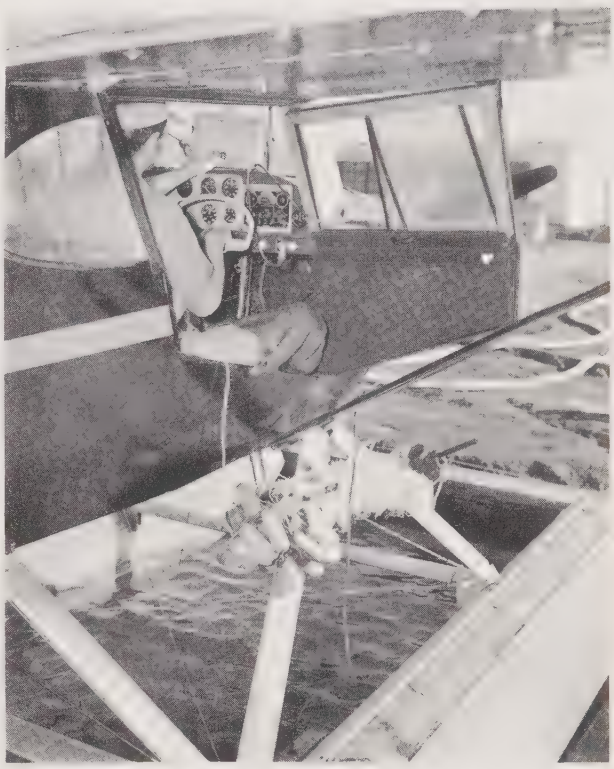
U. S. Fish and Wildlife Service
Anchorage, Alaska

DURING THE PRELIMINARY SURVEYS OF ALASKA'S LAKES, a light aircraft was found to be extremely useful, not only as a means of transportation but also in doing the actual field work. A four-place light aircraft equipped with pontoons was employed in all phases of the survey, which consisted of a general reconnaissance, depth sounding, water sampling, and gill-net setting. The photograph shows the equipment used and its position on the plane.

Before landing on a selected lake, an aerial reconnaissance of the peripheral area was conducted. When flying at elevations of 200 to 500 feet, it was possible to observe readily the number of boats and cabins, the land use, terrestrial vegetation, and terrain. The observer could record in detail such characteristics as natural barriers and beaver activity and could secure general impressions of bottom type, turbidity, aquatic vegetation, and flow of inlets and outlets of the lakes.

After landing, the aircraft functioned as a boat. Aquatic vegetation was collected, bottom types were recorded, and limnological samples obtained. Depth soundings were taken with the aid of a recording fathometer. The recording unit was mounted on top of the instrument panel, the transducer being temporarily attached to a plate permanently mounted on the inboard chine of a pontoon. While the plane was taxiing at slow speed, 550 to 900 revolutions per minute, the transducer crystal was about 6 inches below the surface of the water.



Aircraft with the survey equipment installed. The fathometer can be seen above the instrument panel; the transducer is just beneath the surface of the water. The gill nets are in position for transportation.

Gill nets were transported on specially constructed racks that were attached to pontoon struts. These nets were set by anchoring one end and paying out the remainder as the plane drifted, taking advantage of the wind. This is similar to

Contribution from Dingell-Johnson Project F-1-R, Alaska.

setting a net by boat, but is more dependent on having wind of a suitable direction and velocity. It was possible to move the plane with paddles when there was no wind. When a net was removed from the water, the downwind end was the starting point. Nets were placed directly on the rack.

The drag induced by nets on the outside of the plane reduced cruising speeds by 15 to 20 miles per hour. Stalling speeds were proportionately increased. Work on lakes of marginal size should not be attempted with full equipment aboard.

For lakes of as much as 1,000 acres in area, a reconnaissance of the type that is described here can be completed in 3 to 4 hours, depending on the number of fish taken. Thus, it is possible to complete two lakes daily. This method of survey has decidedly increased the efficiency of Alaska's fresh-water sport-fishery investigations.

MALACHITE GREEN AND GENTIAN VIOLET COMPARED AS TREATMENTS FOR RAINBOW TROUT EGGS

For a number of seasons prior to 1955, a soft-egg condition appeared in the rainbow trout eggs produced at the U. S. Fish and Wildlife Service's fish-cultural station at Manchester, Iowa. In the fall of 1955, there was little evidence of this disease and the general condition of rainbow trout eggs taken from Manchester brood stock was considerably better than in the previous year. Because of continuing difficulty with the disease, however, R. P. Tanner, superintendent, and Harold Newman, fish culturist at the Manchester station, and R. W. Sharp, fishery-management biologist in the Regional Office at Minneapolis, considered it advisable to go ahead with plans to seek a control for the disease. Consequently, they made several experiments to compare the effectiveness of two aniline dyes, malachite green and gentian violet.

In the first experiment, eggs were treated with malachite green flushes (a solution of 1:2,000 applied at the rate of 50 cubic centimeters at the head of the trough and 50 at the middle) and with gentian violet in siphon flows (1:200,000) or in a single dip (1:18,000). The eggs that received the malachite green flushes showed an improved hatch, whereas the eggs treated with gentian violet provided a hatch that was decidedly poorer than the hatch of a group of eggs that received no treatment.

In the second experiment, one group of eggs was treated with a single dip into gentian violet (1:36,000) on the first day and a flush of malachite green on every second day thereafter, whereas another group of eggs was treated with only a flush of malachite green on every second day. The hatches obtained from these groups of eggs were very similar, though there were more crippled fry from the eggs that had been dipped into gentian violet.

In the third experiment, a flush of malachite green was applied to eggs on every second day and gentian violet--as a siphon flow (1:200,000) used every day until eyeing, as a dip (1:36,000) used every day until eyeing, and as a single dip (1:36,000) at 15 hours--was applied to three other groups of eggs. The results obtained from the eggs treated with malachite green were markedly superior to the results obtained from eggs treated with gentian violet.

Owing to the general absence of the soft-egg condition during 1955, its control through the application of either of these dyes was not demonstrated in these tests. The superiority of malachite green as a general egg treatment was indicated, but it is planned to try the effectiveness of gentian violet applied as a simple flush in future trials.

AN INEXPENSIVE SCALE PROJECTOR

Harold K. Hagen
Colorado Agricultural and Mechanical College
Fort Collins, Colorado

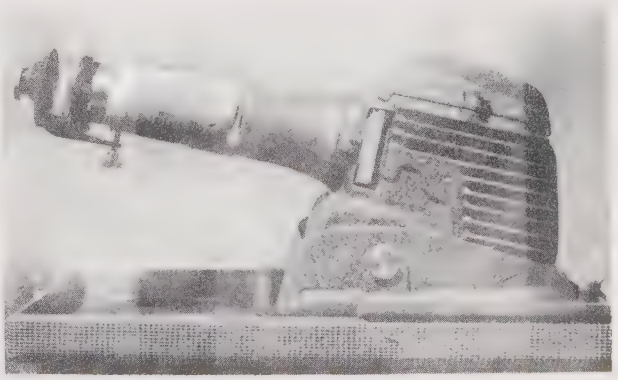
THERE ARE MANY TYPES OF PROJECTION DEVICES currently in use for the reading and analysis of fish scales. Most of these devices are too cumbersome for field use or for use outside a permanent dark room or laboratory. Those units that are portable are generally too costly for wide distribution by budget-minded organizations or commissions. While searching for an inexpensive projection device that could be set up and used in a makeshift laboratory, the author came across the Micro-Beam projection unit manufactured by the S. V. E. Company of Chicago, Illinois.

This small compact unit with a 1-inch f/1.9 Wocoted anastigmatic lens can be used on any projector with a lens 1-43/64 inches in diameter. Where a projector of this size is not available, a simple conversion for almost any type of projector lens tube can be made. A converter sleeve of cardboard and tape can be fashioned (see photograph), and the Micro-Beam can be inserted into one end and held securely by a small thumbscrew on the base of the unit. The other end of the sleeve can be made to slip over a lens tube of almost any size.

The Micro-Beam magnifies 12 times for each foot of projection and, when used horizontally, gives a clear bright image with practically no distortion. Four openings in the metal rotating disk regulate the amount of field covered in projection. The amount of light can be regulated by the size of the bulb used in the projector. A double thickness of the glass slides frequently used in dry-mount scale projection is easily accommodated and firmly held by the spring clip. The Micro-Beam can be used in a semidarkened room, but for best results total darkness is preferable.

In using the Micro-Beam, the author projected a stage micrometer image on a fixed screen and then measured the distance between the projection unit and the screen. A strip of cardboard was placed on the screen, and the projected image of the millimeter scale was marked on the cardboard. The projector could then be dismantled after each reading, as no permanent dark room was available. Thereafter, each time a group of scales was to be projected, the unit could be set up in a matter of minutes by measuring the required distance between the lens and the screen and superimposing the cardboard strip on the projected stage micrometer image to insure uniformity in reading.

The use of the Micro-Beam offered so many advantages that the author persuaded the fisheries division of the South Dakota Department of Game, Fish and Parks to try it. Two units were purchased by that



(Photograph courtesy of the author)

department in 1954, and their value was quickly appreciated. The Micro-Beam is now the standard projection device used by South Dakota.

Of special advantage is the size of the unit. When packaged, it can easily be slipped into a coat pocket. The unit has been used to good advantage in illustrated talks to sportsmen or other audiences that cannot be assembled in laboratories or dark rooms. In 3 or 4 minutes an operator can switch from regular colored slides to the projection of fish scales to emphasize points in fish growth and its importance in management problems.

The Micro-Beam has great possibilities for use with illustrated lectures on other aquatic problems. Whole mounts, temporary mounts, or cross sections can be mounted and shown quickly. For class demonstration and teaching fishery subjects, the Micro-Beam fills an obvious need.

The retail cost of the Micro-Beam is such that most State organizations could easily afford to place a unit with each research crew or at the disposal of any of its personnel who might be called on for quick age analysis or demonstration.

For several years fishery biologists of the Great Lakes Fishery Investigations of the U. S. Fish and Wildlife Service have been working at the Hammond Bay Laboratory near Rogers City, Michigan, to discover a chemical that is selectively toxic to larval sea lampreys. The screening of about five thousand chemicals has revealed several that, in low concentrations, are highly toxic to sea lamprey larvae but are not toxic to other fish.

Among the chemicals screened, a number gave promise of being selectively toxic to various species of game and nongame fish. Thus some chemicals killed goldfish but did not affect trout, whereas other chemicals killed trout but not goldfish. One very interesting chemical killed pumpkinseed and pumpkinseed-bluegill hybrids but did not affect bluegills.

The schooling proclivity of young salmon, and other fishes, is commonly laid to a visual orientation facility. Such a reaction is difficult to measure, but may be readily observed and recorded. To that end a small tank, 3 by 3 feet, was placed in the photographic darkroom of the laboratory, the infrared source and a camera focused on the tank, and the infrared telescope placed handy for observation. The water depth was 4 inches. The school of young silvers . . . formed under the overhead light of the room and was photographed on black and white film. With infrared the only radiation in the room, the school was observed through the telescope. Dispersal was complete in a matter of a few seconds after the overhead light was extinguished. --R. E. Duncan, "Use of Infrared Radiation in the Study of Fish Behavior," Spec. Sci. Rpt., Fish. 170.

To keep nylon rope from unraveling, encircle the ends with a little tape to keep them in place and then hold them over a burner or torch until they have melted and fused together. When the nylon has cooled, it will harden and the remaining tape can be removed.

AN EXPERIMENT WITH VIBERT BOXES

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U. S. Fish and Wildlife Service
Mill Creek, Los Molinos, California

EXPERIMENTAL STREAM PLANTING OF KING SALMON SPAWN has established that stream freshets have an injurious effect on the survival of eggs and alevins. The 1953 work at Mill Creek, California (Gangmark and Broad 1955), verified results of investigators working in other regions. Stream erosion and silting were mentioned as products of flooding which caused damage to eggs in nature.

In 1954, to explore this subject further, Vibert boxes (Vibert 1949) were used, along with the usual plastic mesh sacks, for incubating eggs. The boxes were to serve as a research tool rather than to prove or disprove the Vibert system. As Vibert boxes are made from extremely rigid plastic, the writers felt that damage to eggs by stream erosion could be eliminated. Besides protecting the eggs from the grinding and wearing effect of rock during fast flow of water, the Vibert boxes were expected to protect eggs from the trampling of fishermen and cattle.

This experiment involved plastic sacks and Vibert boxes used under comparable conditions. The plastic sacks--10 by 5.5 inches--were filled with gravel and 100 eggs each; the Vibert boxes were supplied with 100 eggs each. The boxes that were planted in the mainstream of Mill Creek and those that were planted in a channel where the stream flow is controlled for experimental purposes, were first placed inside plastic sacks in order that the alevins emerging from the boxes would be retained for enumeration.

The table and figure show comparisons in percentage of survival from fertilization to the emergent stage. Twenty percent of the samples in the mainstream and the flow-control channel were removed at each of five different stages of development. The percentages of survival for the two types of egg container were similar, though the Vibert boxes were less successful in the last stages. No intermediate sampling was made in the troughs. All samples in the troughs were counted at the emergent stage, 120 days after fertilization. The final production of fry was 0.22 to 0.00 percent, 41.0 to 21.0 percent, and 90.8 to 66.8 percent in favor of the plastic sacks.

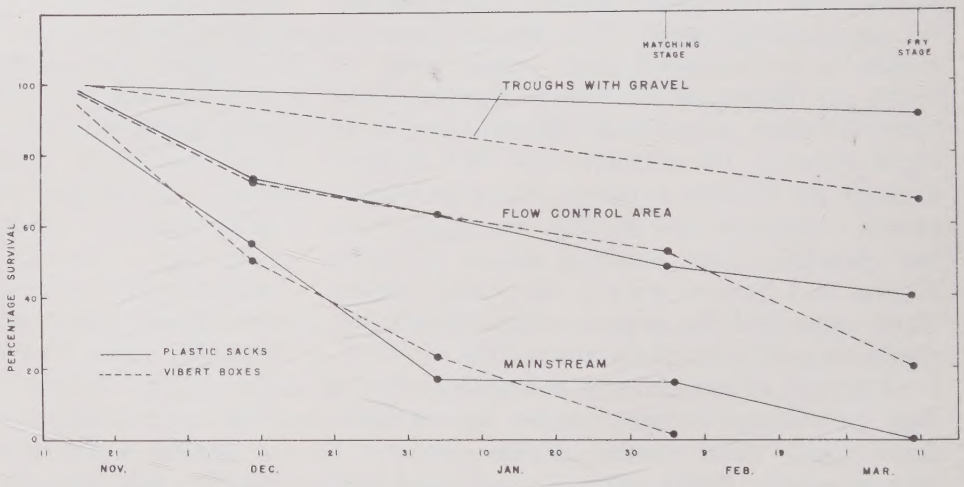
Under circumstances in which erosion removed egg containers from the stream bed, protection by the rigid Vibert boxes was indicated. Three sacks were recovered, December 23, about half a mile downstream, beyond an irrigation diversion dam. Two of the plastic sacks contained Vibert boxes and one contained eggs and gravel that had been planted November 18. The Vibert boxes held 6 and 21 live eggs, respectively, whereas in the third container all were dead.

Much of the time Mill Creek does not qualify as the clear, silt-free type of stream that is recommended for planting. Where there is silting, Vibert recommends using waterproof masks over the boxes or setting the boxes in a mound of gravel above the stream-bed level. The writers think that these methods could not be successfully adopted in Mill Creek because of the erosive nature of the stream. Any influence that the plastic-screen sacks surrounding the Vibert boxes had on normal functioning of the boxes was limited to the mainstream and flow-control channel.

SURVIVAL RATES OF KING SALMON EGGS IN PLASTIC SACKS AND VIBERT BOXES IN THREE EXPERIMENTAL ENVIRONMENTS

Group	Area	Age (days)	Stage	Plastic sacks			Vibert boxes		
				Number of eggs	Number alive	Average percentage survival	Number of eggs	Number alive	Average percentage survival
I	Main-stream	3	Green	2,000	1,785	89.20	1,000	944	94.4
	Flow-control	3		1,600	1,569	98.10	500	491	98.2
II	Main-stream	29	Eyed	1,200	663	55.20	900	460	51.1
	Flow-control	29		1,600	1,177	73.60	500	363	72.6
III	Main-stream	54	Eyed	1,400	236	16.80	800	188	23.5
	Flow-control	54		1,600	1,014	63.40	500	317	63.4
IV	Main-stream	86	Hatch	1,700	276	16.20	500	9	1.8
	Flow-control	86		1,600	777	48.60	500	263	52.6
V	Main-stream	119	Fry	900	2	.22	500	0	.0
	Flow-control	119		1,600	641	40.10	500	105	21.0
	Trough 1	120	Fry	1,000	865	86.50	1,000	616	61.6
	Trough 2	120		1,000	950	95.00	1,000	719	71.9

Survival rates for king salmon eggs planted in plastic sacks and Vibert boxes in three experimental areas.



Those buried in the gravel-filled troughs were planted in the normal way.

It had been supposed that use of the rigid boxes, rather than plastic sacks, would eliminate the grinding, wearing factor of stream erosion; but, except for three samples washed downstream, results favored the sacks.

King salmon alevins apparently had no trouble in escaping through the slots in the Vibert boxes.

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☆ U. S. GOVERNMENT PRINTING OFFICE : 1956 - 361704

UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Washington 25, D. C.

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